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DETECTION AND SIGNIFICANCE OF MANGANESE DIOXIDE IN THE SOIL

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Work on the determination of organic matter in soils by means of hydrogen peroxide revealed that a sample of Blakely loam decomposed the hydrogen peroxide so rapidly that the organic matter was not destroyed. The soil contained an unusual quantity of manganese and had a peculiar, dark brown color. Preliminary tests indicated that the Blakely loam and certain lateritic soils having a similar color, high manganese content, and high hydrogen peroxide activity contained manganese in the dioxide form.

This study was undertaken to determine whether the hydrogen peroxide activity of a soil could be taken as a positive indication of the presence of manganese dioxide, and if so, to study the distribution of manganese dioxide in some of the soil series.

PREVIOUS WORK ON THE OCCURRENCE OF MANGANESE DIOXIDE IN SOILS

Concretions of manganese dioxide have been noted in the soil by Doherty (6), Thresh (28), and Helbig (11). Worsham et al. (31) have reported the presence of manganese dioxide in the dark colored Davidson Clay in Georgia. Manganese dioxide in large quantities has been reported in Hawaiian soils by Kelly (13). McGeorge (15) and Johnson (12) have reported further occurrences of manganese dioxide in Hawaiian soils. Johnson gives some quantitative data based on the determination of the oxygen evolved on heating the soil. Bertrand (2) makes the general statement that manganese occurs in the soil in several different forms, including the dioxide. Sharrer (25) has shown that there is a correlation between the catalytic power of a soil and the manganese and iron content.

HYDROGEN PEROXIDE TEST FOR MANGANESE DIOXIDE IN THE SOIL

Platinum black, metallic peroxides, charcoal, chromium trioxide, and manganese dioxide decompose hydrogen peroxide with great energy. Of all these substances, manganese dioxide is the only one likely to be of any considerable occurrence in soils.[1]

[1] It has been stated that ferric oxide will decompose hydrogen peroxide rapidly. Much depends upon the samples of ferric oxide used. Many red soils containing as high as 34 per cent Fe_2O_3 will not decompose hydrogen peroxide rapidly.

It has been stated also that charcoal is a common constituent of soils. It is only rarely, however, that the quantities involved are sufficient to cause rapid decomposition of hydrogen

TABLE 1

Relation between total manganese and capacity of the soil material to decompose hydrogen peroxide

SOIL	DEPTH	TOTAL MANGANESE AS MnO	RATE OF DECOMPOSITION OF H_2O_2
	inches	*per cent*	
Blakely loam, Georgia*	0–15	0.50	200 cc. in 6 minutes
	15–24	0.22	200 cc. in 20 minutes
	24–48	0.10	30 cc. in 30 minutes
	48–60	0.03	No activity
	60–96	0.37	200 cc. in 18 minutes
	96–116	0.38	200 cc. in 12 minutes
Blakely clay loam, Georgia	0–3	0.68	200 cc. in 9 minutes
	3–10	0.84	200 cc. in 7.9 minutes
	10–22	0.52	185 cc. in 30 minutes
	22–45	0.19	Very little activity
	45–70	0.17	Practically no activity
Blakely clay loam, Georgia	0–1½	0.53	200 cc. in 10 minutes
	1½–7	0.49	200 cc. in 17 minutes
	7–20	0.33	200 cc. in 24 minutes
	20–45	0.15	30 cc. in 30 minutes
	45–60	0.53	200 cc. in 11 minutes
Blakely clay loam, Georgia	0–½	1.20	200 cc. in 2.9 minutes
	½–3	1.27	200 cc. in 2.5 minutes
	3–15	0.89	200 cc. in 3.3 minutes
	15–36	0.50	200 cc. in 28.0 minutes
Decatur silt loam, Ala*	0–3	0.23	95 cc. in 30 minutes
	3–6	0.28	88 cc. in 30 minutes
	6–10	0.15	82 cc. in 30 minutes
	10–18	0.03	5 cc. in 30 minutes
	18–40	0.03	7 cc. in 30 minutes
	40–70	0.02	4 cc. in 30 minutes
Dicklom loam, Philippines	0–6	0.57	130 cc. in 30 minutes
	6–36	0.23	85 cc. in 30 minutes
	36–48	0.18	55 cc. in 30 minutes
Greenville sandy loam, Alabama*	0–4	0.17	20 cc. in 30 minutes
	4–48	0.04	No activity
	48–78	2.85	200 cc. in 1.7 minutes
	78–114	0.03	No activity
Greenville sandy loam, Georgia	0–2	0.09	Practically no activity
	2–9	0.05	Practically no activity
	9–40	0.02	Practically no activity
	40–52	0.03	Practically no activity
	52–55	1.02	200 cc. in 21 minutes
	55–70	0.02	No activity

* Analysis for MnO by G. Edgington, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

TABLE 1—*Continued*

SOIL	DEPTH	TOTAL MANGANESE AS MnO	RATE OF DECOMPOSITION OF H_2O_2
	inches	*per cent*	
Hagerstown loam, Tennessee	0–4½	0.44	137 cc. in 30 minutes
	4½–11	0.37	110 cc. in 30 minutes
	11–24	0.27	80 cc. in 30 minutes
	24–36	0.34	50 cc. in 50 minutes
Lurugan loam, Phillippines	0–8	0.80	200 cc. in 11 minutes
	8–36	0.44	170 cc. in 30 minutes
	36–48	0.43	Little activity
Marshall silt loam, (Phase of) Kansas	0–13	0.12	30 cc. in 30 minutes
	13–25	0.13	35 cc. in 30 minutes
	25–32	0.16	63 cc. in 30 minutes
	32–54	0.19	55 cc. in 30 minutes
	54–62	0.11	33 cc. in 30 minutes
	62+	1.03	200 cc. in 0.5 minute
Summit silt loam, Kansas	0–8	0.07	15 cc. in 30 minutes
	8–14	0.12	25 cc. in 30 minutes
	14–26	0.27	50 cc. in 30 minutes
	26–48	0.32	200 cc. in 11 minutes
	48–72	0.19	115 cc. in 30 minutes

The power of a number of soils to decompose hydrogen peroxide and the manganese content of these soils have been compared. For this purpose profile samples of the soil were taken. One gram of the soil was put in a flask connected with a gas burette, 10 cc. H_2O and 10 cc. 30 per cent H_2O_2 were introduced, and the gas evolved in 30 minutes, or the time necessary to evolve 200 cc. was measured. The flask was shaken every minute. The peroxide used was Merck's "Superoxol." It was only very slightly acid and contained some preservative. It was not made neutral or slightly alkaline with NaOH as is done in soil studies where the activity of the soil catalase is involved.

The results of the test, given in table 1, show that high manganese content of the soil is associated with high hydrogen peroxide activity.

The question arises as to what combination of manganese is responsible for the very rapid decomposition of the peroxide. From the sandy fractions of all the samples given in table 1 which showed a hydrogen peroxide activity of 200 cc. in less than 30 minutes, a number of black or dark brown concretions could be separated. These concretions would decompose hydrogen peroxide

peroxide. Of about fifty soils which contained charcoal, only one contained enough to decompose hydrogen peroxide rapidly. This soil was from the "charcoal plot" at the Pennsylvania experiment station. It contained about 5 per cent charcoal and was once the site of a charcoal furnace.

vigorously, particularly when finely ground. Further, if small quantities of the sandy fractions were scattered loosely on the bottom of a beaker and covered with dilute H_2O_2, the origin of the arising bubbles could invariably be traced to the black concretions. Samples of hand picked concretions yielded on analysis from 8.28 per cent MnO_2 in the Scott silt loam to 34 per cent in the Blakely loam. These concretions gave off oxygen when heated and caused chlorine to be given off from hydrochloric acid. There can be no doubt that the concretions were impure manganese dioxide. For the sake of brevity, these concretions will be called "manganese concretions." They commonly contain much more ferric oxide and other soil material than they do manganese dioxide.

TABLE 2

Relation of total manganese in soil fractions to the power to decompose hydrogen peroxide

SOIL AND FRACTION	MnO	RATE OF DECOMPOSITION
	per cent	
Blakely loam, 16–24 inches:		
Sand	0.21	100 cc. in 30 minutes
Silt	1.23	200 cc. in 1.5 minutes
Colloid	0.54	23 cc. in 30 minutes
Greenville sandy loam, 48–78 inches:		
Sand	1.87	200 cc. in 1.4 minutes
Silt	4.93	200 cc. in 0.1 minute
Colloid	1.02	38 cc. in 30 minutes
Orangeburg fine sandy loam, 10–36 inches:		
Sand	0.18	Very little activity
Silt	0.67	55 cc. in 30 minutes
Colloid	0.59	30 cc. in 30 minutes
Clarksville silt loam:		
Colloid	0.37	30 cc. in 30 minutes
Manor loam:		
Colloid	0.11	7 cc. in 30 minutes
Susquehanna clay:		
Colloid	0.30	5 cc. in 30 minutes
Vega Baja clay loam:		
Colloid	0.16	15 cc. in 30 minutes

W. H. Fry, of this Bureau, reported that the sands and silts from the Blakely and Greenville surface soils contained no silicates or carbonates of manganese that could be discovered petrographically by using a reasonable quantity of sample. There is very little probability of manganese silicates or carbonates being present in such highly weathered soils as the Blakely and Greenville. Finely ground manganese silicates do not decompose hydrogen peroxide.

It seems pretty well established that manganese concretions were responsible for the hydrogen peroxide activity of the sandy fractions of the samples given in table 1. Further light might be shed on the problem by studying the

manganese content and hydrogen peroxide activity of the sand, silt, and clay fractions. These separations were made in the case of three soils. The data are given in table 2, together with the data of a number of soil colloids containing considerable manganese.

The data given in table 2 show that the silt fractions were the highest in manganese and the most active in decomposing H_2O_2. This is in harmony with the studies of McGeorge (15), who found that the silt and very fine sand fractions of a number of Hawaiian soils contained the most manganese and that the manganese was largely in the form of manganese dioxide.

It is surprising that the clay, having a relatively enormous surface, and a comparatively high manganese content, should be so inactive in decomposing hydrogen peroxide. The finer manganese dioxide is ground the more readily will it decompose hydrogen peroxide. Very small quantities of finely ground manganese dioxide when added to the inactive colloids will make them very active in decomposing hydrogen peroxide. We must conclude that the soil colloids examined did not contain manganese dioxide.

TABLE 3

Exchangeable manganese in fractions of Blakely loam and Greenville sandy loam

SOIL	DEPTH	FRACTION	EXCHANGEABLE MANGANESE AS MnO	TOTAL MANGANESE AS MnO
	inches		*per cent*	*per cent*
Blakely loam	16–24	Sand	0.046	0.21
		Silt	0.069	1.23
		Colloid	0.410	0.54
Greenville sandy loam	48–78	Sand	0.042	1.87
		Silt	0.075	4.93
		Colloid	0.330	1.03

For the purpose of throwing some light on the question of the forms of manganese present in the different mechanical soil fractions, the sand, silt, and clay fractions of the Blakely and Greenville soils were tested for exchangeable manganese. One gram samples were washed on a filter paper with 250 cc. of normal ammonium chloride. The manganese was determined in the filtrate. The results of the study are given in table 3.

The data given in table 3 show that a considerable part of the manganese in the colloidal matter is exchangeable while comparatively little of the manganese in the silts and sands is exchangeable. A large part of the manganese in the sands and silts seems to be in the form of the dioxide. Since fine grinding of manganese dioxide increases its activity, and the clay which is very finely divided, contains relatively large quantities of manganese, it is plain there can be little manganese dioxide in the clay. The fact that the manganese in the clay is partly exchangeable and not in the form of manganese dioxide would lead to the belief that the manganese in this fraction occurs as a part of the basic radical of the absorption complex.

QUANTITATIVE ASPECT OF THE HYDROGEN PEROXIDE TEST FOR MANGANESE DIOXIDE

Positive identification of manganese dioxide in the soil is very desirable since the occurrence of manganese dioxide seems to be characteristic of certain series of soil. The determination should be quantitative, if possible, for there is presumably some relation between the quantity of manganese dioxide and certain properties of the soil.

The quantitative methods for the determination of manganese dioxide in ores fail, in general, in the case of soils. This is due to the almost universal presence of organic matter, ferrous iron minerals, and, occasionally, calcium carbonate. The Bunsen method, in which the manganese is estimated by measuring the quantity of chlorine liberated by boiling with hydrochloric acid, would be accurate were it not for the interference of ferrous iron minerals and organic matter and also the apparent very slow liberation of chlorine from the boiling soil solution when no manganese dioxide is present. The evolution of a considerable quantity of chlorine at the first boiling of the soil solution is a positive indication of manganese dioxide.

Brewer and Carr (3) report some quantitative determinations of manganese dioxide in the soil. They modified the Bunsen method (26) by adding mercuric oxide to prevent the chlorination of the organic matter. Their results on synthetic mixtures of soils and considerable quantities of manganese dioxide showed that under these conditions nearly all the manganese added could be detected. The results in the case of two soils were 0.16 per cent MnO_2 too low.

Brewer and Carr recommend a method employing an acid solution of ferrous ammonium sulfate to dissolve the manganese dioxide in the soil. They recognize two forms of manganese, the easily soluble manganese and manganese dioxide. The easily soluble manganese is determined by digesting with 1.1 per cent sulfuric acid. The soil is then digested with a mixed solution containing 1.1 per cent sulfuric acid and 5 per cent ferrous ammonium sulfate. The difference between the manganese dissolved by the mixed solution and that dissolved by the 1.1 per cent sulfuric acid is considered to be manganese dioxide.

Brewer and Carr's method apparently fails with some soils, particularly in soil colloids containing a considerable quantity of manganese. In these cases the acid ferrous ammonium sulfate dissolves considerably more manganese than the sulfuric acid solution alone, although manganese dioxide is apparently not present in soil colloids.

The method employed by Johnson (12) is applicable only to soils free from organic matter. In this method the soils are heated to redness, and the oxygen evolved during heating is a measure of the quantity of manganese dioxide present

Work with synthetic mixtures of various soils with manganese dioxide has

shown that none of the proposed methods are reliable for the determination of manganese in the form of dioxide. Ferrous iron minerals and organic matter may mask as much as as 1 per cent of manganese in methods where the chlorine evolved is taken as a measure of manganese dioxide.

Tests in the foregoing synthetic mixtures have shown the hydrogen peroxide test will detect one-tenth per cent of manganese dioxide in soils. With these synthetic mixtures the rate at which the peroxide is decomposed is proportional to the quantity of manganese dioxide present. This suggested that the test might be quantitative.

It was first established that the rates of oxygen and heat evolution from a mixture of hydrogen peroxide and a soil containing no manganese dioxide but much organic matter were negligible compared to the rate of decomposition when manganese dioxide was present. This statement seemingly contradicts numerous experiments in the measurement of soil catalysis. The conditions are different. In soil catalysis experiments reported by May and Gile (18) and by Sharrer (25) the hydrogen peroxide is comparatively weak and it is

TABLE 4

Time required to reach a maximum temperature and to liberate 1000 cc. oxygen with varying quantities of psilomelane

QUANTITY OF PSILOMELANE	TIME TO REACH MAXIMUM TEMPERATURE	TIME TO EVOLVE 1000 CC.	RISE OF CALORIMETER CONTENTS
gm.	*minutes*	*minutes*	*°C.*
0.10	230	62	27.7
0.15	97	40	28.0
0.25	45	21	28.0

made just barely alkaline with sodium hydroxide. The hydrogen peroxide from such a solution is rapidly decomposed by most highly organic soils and particularly by partially decayed banana peels. Under these conditions the decomposition is rapid in soils containing no manganese dioxide. Loew (14) has shown, however, that when strong hydrogen peroxide such as Merck's "Superoxol" is used the catalase is apparently destroyed. "Superoxol," diluted with its own volume of water is neutral to litmus paper. The decomposition rate of this diluted "Superoxol" by a very fertile garden soil or by banana peel is negligible compared to the decomposition rate when manganese dioxide is present.

To determine the quantitative possibilities of the hydrogen peroxide test, varying quantities of finely ground psilomelane were mixed with 5 gm. of soil showing no reaction with the peroxide and added to 20 cc. 15 per cent hydrogen peroxide in an adiabatic calorimeter. The gas and heat given off were measured and the time noted necessary to reach 1000 cc. gas and the maximum temperature. The data are given in table 4.

The data of table 4 show that the rates of heat and oxygen evolution are

dependent upon the quantities of manganese dioxide present. They also show that the measure of the rate of oxygen evolution is more practical than that of heat evolution.

If the same conditions were present in soils, particularly as to the extent of subdivision of the manganese dioxide, as were present in the experiment just described, one would expect that the method might be used to determine the quantity of manganese dioxide in the soil. The varying surface, however, complicates the case. It has been shown that manganese dioxide occurs in the sands and silts only, and not in the clay. With the elimination of the clay, the chances for great variation in the surface of any particular percentage of manganese dioxide are somewhat restricted. It is hardly to be supposed that there will be an equal size distribution of the manganese dioxide particles in the sands and silts of various soils. If there is not an even size distribution and if the rate at which hydrogen peroxide is decomposed varies very much

TABLE 5

*Relation between size of psilomelane particles and hydrogen peroxide activity**

SIZE OF PSILOMELANE PARTICLES	TIME NECESSARY TO GIVE 1000 CC.	TIME NECESSARY TO REACH MAXIMUM TEMPERATURE
mm. diameter	*minutes*	*minutes*
0.50–0.33	178	218
0.084–0.01	25	4.5
0.01	Too rapid to measure	Too rapid to measure

* For 1 gm. psilomelane. 10 cc. H_2O_2 and 10 cc. H_2O.

with the size of the manganese dioxide particles, then the method can not be used for a quantitative determination of the manganese dioxide present.

Some experiments on the variation of the rate at which hydrogen peroxide is decomposed by various sized particles of manganese dioxide have been made. A sample of psilomelane was ground and sieved into three sizes. The rates at which 1 gm. of each of the sizes decomposed 20 cc. of 15 per cent hydrogen peroxide were measured. The results are given in table 5.

The data given in table 5 show that the state of subdivision has a great deal to do with the rate at which manganese dioxide decomposes hydrogen peroxide. The effect of varying the sizes of the manganese dioxide on the quantitative value of the hydrogen peroxide activity can be seen by assuming that all the manganese dioxide particles in a certain soil A are from 0.50-0.33 mm. diameter and in soil B, from 0.084–.01 mm. diameter. Suppose A and B give identical rates of decomposition of the hydrogen peroxide solution, soil A would have many times more manganese dioxide than soil B. Such differences as the above in size distribution are probably extreme and would not occur in natural soils.

APPROXIMATE METHOD FOR THE DETERMINATION OF MANGANESE DIOXIDE IN SOILS

Johnson (12) has shown that nearly all the manganese in Hawaiian soils high in that element, is in the form of manganese dioxide. Our results show that the weathered manganese is in two forms in soils containing manganese dioxide. A part of it is absorbed by the colloidal complex, the rest is apparently in the form of the dioxide in the sands and silts. Manganese dioxide in soils is a product of rock decay or rock alteration and if the conditions in the soil are such that any manganese dioxide is formed, it is probable that all the manganese in the sands and silts would be in the form of the dioxide. Based on the above assumptions, two methods are suggested for the determination of manganese dioxide in the soil. The hydrogen peroxide test is first used to establish the presence of the dioxide.

The presence of manganese dioxide having been established by the hydrogen peroxide test, the soil may be separated into colloidal and non-colloidal fractions and the total manganese determined in the non-colloidal fraction. According to the assumption all the manganese in the sand and silt is in the form of the dioxide. The chlorine evolved rapidly by the Bunsen method checked fairly well with the total manganese in the sands and silt of the Blakely and Greenville soil materials in which much manganese was present. Another way would be to determine the total manganese and the exchangeable manganese in the whole soil and the difference should represent the maximum quantity of manganese present as manganese dioxide. Not all the manganese in the colloidal matter is exchangeable, it is true, but if the colloidal matter is low and the total manganese is high, the error due to this cause is lessened. Brewer and Carr's (3) method is, however, more advisable when the colloidal matter has not been separated from the non-colloidal matter.

There is little probability of manganese existing as a silicate in the sands and silts of soils containing manganese dioxide. It has been noted that two manganiferous soils did not contain any manganese silicate that could be detected petrographically. In general, any soil containing manganese dioxide is so thoroughly weathered that there is very little likelihood of any undecomposed manganese silicate being present.

In this work it has been shown that manganese dioxide particles could be separated from all soils (about 125 samples) which evolved more than 200 cc. oxygen in 30 minutes from a mixture of 1 gm. of finely ground soil, 10 cc. of "Superoxol," and 10 cc. of water. Occasionally a sample giving only slightly less than 200 cc. has contained a concretion of impure manganese dioxide. On the other hand, an occasional sample has given nearly 200 cc. under like condition, and from these samples no manganese dioxide could be separated. The data shown in table 1 do not indicate a proportionality between the total manganese and the rate of oxygen evolution. Without reasoning in a circle it is impossible to tell how the total manganese is divided between the colloid and

manganese dioxide. However, the rate of oxygen and heat evolution will tell whether there is little, considerable, or a relatively large quantity of manganese present as the dioxide. Although the rate of oxygen evolution of a synthetic mixture of soil and manganese dioxide is dependent on the quantity of manganese dioxide, it has been shown that the rate is dependent upon the extent to which the manganese dioxide is subdivided. The size of the manganese dioxide particles in different soils may not vary greatly, but there is too much uncertainty to assume that they do not. It seems better, therefore, to rely on the hydrogen peroxide test as a qualitative indication of the presence of manganese dioxide and determine the total manganese in the sands and silts or use Brewer and Carr's method, knowing that the quantity of dioxide shown is somewhat greater than that actually present.

SOLUTION OF MANGANESE AND DEPOSITION OF MANGANESE DIOXIDE

Manganese occurs in the lithosphere in very small quantities. Clarke (4) gives the average as 0.11 per cent MnO. The numerical average for soils is about 0.12 per cent, the range being from less than 0.001 per cent to 1.27 per cent in surface soils of the United States. Tropical soils may contain as high as 15 per cent.

Manganese is present in the manganous condition as a minor constituent of igneous rocks. When the rock weathers the manganese is released rather easily, resembling ferrous iron in this respect (19).

Manganese is dissolved and transported as manganous bicarbonate (21) sometimes to a great distance. Chalybeate waters containing both iron and manganese deposit their iron sooner than manganese according to Clarke (4). This property, together with the fact that the manganese content of the lithosphere is small, possibly accounts for the occurrence of relatively few and concentrated deposits of manganese and also for the localized distribution of manganese in the soil profile.

The dissolved manganese in river, spring, and well waters varies greatly. It is generally only a trace but reaches as high as 5 to 10 p.p.m. in carbonated waters (4).

The solubility of manganese carbonate in carbon dioxide waters is given by Gmelin-Kraut (10) as 0.130 gm. per liter and by Ageno and Valla (1) as 0.065 gm. per liter at 25°. According to Roscoe and Schorlemmer (22) manganese carbonate dissolves in water saturated with carbon dioxide to the extent of 0.25 gm. per liter.

Manganese is particularly susceptible to solution and deposition under soil conditions, because of the readiness with which it assumes different states of oxidation and the different solubilities of manganous and manganic compounds. When in the manganous state it is soluble to the extent of several parts per million in meteoric waters. Manganous salts are more stable under atmospheric conditions than are ferrous salts. Manganous manganese exists in the colloidal matter of well-drained surface soils even when the reaction is only slightly acid.

Although manganous solutions are more stable than ferrous iron solutions, they are readily oxidized by various natural agencies to the insoluble dioxide. Murray and Irvine (20) report an example of this oxidation in the Clyde River in Scotland. The headwaters contain manganous bicarbonate in solution. Near the mouth of the river the pebbles near the bank are coated with black manganese dioxide, and suspended particles of manganese dioxide may be detected in the water. In municipal water supply systems manganese bearing waters frequently give trouble by clogging the water mains with precipitated manganese dioxide.

Manganese dioxide is practically insoluble in water. Under certain soil conditions it is very stable and the composition of some lateritic soils would lead to the belief that manganese dioxide is less soluble than iron and aluminum oxides. Although manganese is present in the parent rock in very small quantities and iron and aluminum are present in very large quantities, the manganese may make up several per cent of the lateritic soil.

The solibiluty of manganese in the soil is greatly dependent upon the reaction of the soil and upon whether oxidizing or reducing conditions prevail. Ruprecht and Morse (23) have shown that manganese is one of the easily replaceable bases, and Mattson (17) has shown that it is replaced most easily when the reaction is decidedly acid. This is in line with field experience. Ruprecht and Morse (23) found that the manganese was very soluble in certain Massachusetts plots made acid by the long continued application of ammonium sulfate. Kelly and McGeorge (13) show that the manganese is made more soluble by heating the soil, particularly when the soil is heated high enough to reduce the manganese by the organic matter. In this laboratory it has been found that manganese becomes very soluble in water-logged soils. The solubility of the manganese in a number of air-dried soils from nearby Maryland and Virginia as determined by shaking the air-dried soil with water and letting settle over night, was less than one part per million. When the soil was submerged in water in a stoppered bottle for several days the soluble manganese rose as high as 500 p.p.m. calculated on the weight of the soil.

The conditions which bring about the precipitation of manganese dioxide, though not well known, must be very sharply defined, because very pure deposits are found in media containing only traces of manganese. Large quantities of manganese nodules are found on the surface of certain deep sea deposits. In some soil profiles it is conspicuously absent from some layers but occurs in other layers in large quantities.

Penrose (21) states that manganese replaces calcium in calcium carbonate and Murray and Irvine (20) advance this hypothesis to account for the formation of manganese nodules around calciferous organic remains.

Weidman (32) reports a pseudomorph of pyrolusite after calcite from northern Wisconsin. Here is a clear case of calcium carbonate precipitating manganese dioxide.

If solutions of manganous bicarbonate are percolated through columns of

calcium carbonate and the percolation is not continued too long, the manganese is almost quantitatively precipitated. After drying the calcium carbonate, dark particles, presumably manganese dioxide, can be detected in the mass. These dark particles decomposed hydrogen peroxide energetically.

Geloso (8) has found that colloidal manganese dioxide has the power to adsorb manganous salts. Tillmans, Hirsh, and Haffner (29) describe a process for the removal of manganese salts from water supplies. The water is filtered through manganese dioxide, which adsorbs the manganous salts, changing them into the insoluble dioxide. The formation of manganese concretions may be explained in this way. Once a particle of manganese dioxide is formed through the replacing of calcium, precipitation by bacteria (7), or any other cause, such a particle would continue to grow by adsorbing and fixing the manganous salts within its sphere of action.

In the soil, there are instances where the artificial addition of calcium carbonate has precipitated manganese. Schollenberger (24) found a black precipitate containing manganese on the surface of calcium carbonate particles picked out from pots of soil that had recently been limed. On some of the limed plots at the Rhode Island station plants suffer chlorosis which is supposed to be due in this case to lack of manganese (9). The precipitation and change of the exchangeable manganese to the insoluble dioxide would seem to be the explanation of this phenomenon. According to Truog (30) Masoni (16), Ruprecht and Morse (23), and Funchess (7), liming the soil makes the manganese insoluble or nearly so.

In the development of some soil profiles there has been a concentration of manganese in certain horizons. As a general rule, manganese is highest in the surface soil. It reaches a minimum in the B horizon and generally increases in the C horizon. Oxidation would favor the precipitation of manganese but in most soils manganese dioxide is not present. The manganese is possibly concentrated in the surface soil through the agency of plants. In many soils containing a layer of calcium carbonate below the surface, deposits of manganese dioxide are found just above the layer containing calcium carbonate. These deposits occur as well-defined concretions, as leaf-like deposits in soil cracks, and as a loose black powder in cavities formerly filled by calcium carbonate.

There is an interesting relation between the total manganese and the reaction of the horizons in soil profiles containing manganese dioxide. These relations are given for a few profiles in table 6.

The Blakely series in which manganese dioxide is present in the surface soil shows a correlation between the total manganese and the pH. In those soils the pH and percentage of manganese pass through a minimum in the B horizon and no manganese dioxide is found in the B horizon. A number of profile samples show the lack of manganese dioxide in the B horizon, but only two profiles, those shown in the table, extend into the C horizon. The Blakely series was originally formed from limestone but is deeply weathered and there

is no calcium carbonate in the soil profile. The results with the Blakely indicate that manganese dioxide is dissolved in the soil when the pH is below 5.0 or 5.2.

TABLE 6

Showing the relation between total manganese and pH in the soil material of certain profiles

SOIL SERIES	SOIL NUMBER	DEPTH	MnO	pH*
		inches	*per cent*	
Blakely clay loam, Daugherty Co., Ga.	30213	0–15	0.501	6.07
	30214	15–24	0.224	6.45
	30215	24–48	0.103	5.13
	30216	48–60	0.026	5.50
	30217	60–96	0.367	7.35
	30218	96–116	0.383	7.55
Blakely clay loam, Early Co., Ga.	32457	0–1½	0.53	6.87
	32458	1½–7	0.49	5.37
	32459	7–20	0.33	5.33
	32460	20–45	0.15	4.94
	32461	45–60	0.53	5.06
Blakely clay loam, Randolph Co., Ga.	258214	0–½	1.20	7.00
	258215	½–3	1.27	6.50
	258216	3–15	0.89	5.88
	258217	15–36	0.50	5.34
Labette silty clay loam, Crawford Co., Kan.	3821A	0–9	0.03	5.15
	B	9–14	0.05	5.13
	C	14–25	0.35	5.03
	D	25–35	0.30	5.19
	E	35–44	0.40	6.45
	F	44–60	0.09	7.75
Marshall silt loam, Doniphan Co., Kan.	3819A	0–13	0.12	5.88
	B	13–25	0.13	6.00
	C	25–32	0.16	6.09
	D	32–54	0.19	6.36
	E	54–62	0.11	7.80
	F	62+	1.03	7.79

* The pH determinations were made by E. H. Bailey of this Bureau.

The results on the Labette sample show that manganese has been carried down from the acid surface, but the precipitation apparently takes place in an acid horizon.

Well-defined manganese concretions occur in the Hockley and Edna silt loams in Victoria County, Texas. The concretions are found just above the carbonate layer, which is about 4 feet deep. The pH of the surface layers is about 6, rising to 7 and 8 where the concretions occur.

The Miami series, Miami County, Indiana, occasionally but not always show a few scale-like precipitates or soft concretions of manganese dioxide 3 or 4 feet below the surface. In this series the pH varies in the surface layers from 4 to 6 and reaches about 8 in the layer where the manganese occurs. Conrey and Schollenberger's (5) data show a similar correlation between manganese content and pH in the Clermont silt loam in Clermont County, Ohio.

SIGNIFICANCE OF MANGANESE DIOXIDE IN THE SOIL

Soils which have manganese dioxide in the A horizon have distinctive properties. They are rather sharply defined from contiguous soils containing no manganese dioxide. The color, a peculiar chocolate brown, is distinctive and unmistakable. The soil is loose, friable, well-drained, and in Hawaii generally contains less clay than adjoining soils (13). Pineapple plants growing on soils high in manganese dioxide frequently show chlorosis and many other plants do not develop normally on these soils. In our southern states, however, the Blakely soils, which are characterized by the manganese dioxide they contain, are markedly fertile.

Worsham et al. (31) have reported the presence of manganese dioxide in the "push land," a phase of the Davidson clay in Georgia. A great number of soils were tested for the presence of manganese dioxide with hydrogen peroxide.[2] All surface soils showing the presence of manganese dioxide had a peculiar chocolate brown color. These surface soils were confined to a very few series. Of these the Blakely series in Georgia and Alabama stood out prominently. It was found that this laboratory test would distinguish the Blakely from the contiguous Greenville and Orangeburg series. Some samples of the Davidson and Decatur surface soils gave the test. Lateritic soils from the Philippines, East Indies, and Cuba showed the presence of manganese dioxide in the surface soil.

Scale-like deposits and occasional concretions of manganese dioxide are occasionally found just above the horizon which contains limestone, as in the Fox, Miami, Bellefontaine, Clermont, Fillmore, and Vigo series. In general, however, the soil material in this particular horizon of the series as noted above does not give a positive test for manganese dioxide.

Well-defined concretions containing manganese dioxide occur in the lower horizons of the Hockley and Edna series in Texas and in the Scott series in Nebraska. These concretions occur just above the profile containing calcium carbonate.

In a phase of the Marshall silt loam in Troy, Kansas, 5 feet below the surface, manganese dioxide was present as a black, loose powder in cavities where limestone had been leached out.

Two profiles of the Greenville series showed relatively rich concentrations of manganese dioxide from 4 to 6 feet below the surface. The dioxide was

[2] Through the coöperation of Mark Baldwin, H. H. Bennett, W. T. Carter, W. E. Hearn, T. D. Rice, R. S. Roberts, and W. E. Tharp of the Soil Survey.

not present in well-defined concretions. One horizon contained 2.85 per cent MnO, whereas horizons just above and below contained only 0.03 per cent MnO. No calcium carbonate appears in the lower horizons of this series, but the series is considered as being derived from limestone and the precipitation of the manganese dioxide was probably caused by calcium carbonate.

Manganese concretions are found in the upper part of the Decatur profiles in northern Alabama. In the Shelbyville series in Kentucky, soft manganese concretions in the upper part of the C horizon are a characteristic feature of the series.

Two rough analyses of the colloidal matter from the Blakely series show a $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio of slightly less than 0.8. The analyses of lateritic soils containing manganese dioxide indicate a low $\frac{SiO_2}{Al_2O_3 + Fe_2O_3}$ ratio in the colloidal matter. A low silica-alumina-iron ratio is undoubtedly a characteristic of the colloidal matter in surface soils containing manganese dioxide.

SUMMARY

The presence of a small quantity of manganese dioxide in the soil causes the soil to decompose hydrogen peroxide so vigorously that the presence of manganese dioxide in the soil can be established by the hydrogen peroxide test. The test is not recommended for a quantitative determination of manganese dioxide, on account of probable differences to be found in the sizes of the manganese dioxide particles in different soils.

Manganese dioxide does not occur in the clay or colloidal fractions of soil. It occurs in the sands, but to a greater extent in the silt fraction. The determination of the total manganese in the silts and sands is an approximate determination of the manganese dioxide in the whole soil.

Concretionary and other deposits of manganese dioxide in the soil are apparently formed through the agency of calcium carbonate.

The presence of manganese dioxide in the surface layers of soil is characteristic of certain soil series. These soils are characterized by a peculiar and unmistakable chocolate brown color.

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AN EFFICIENT SOIL TUBE JACK

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During the past year the authors[1] have been conducting soil moisture studies is southern California, which require soil samples taken at 1-foot intervals to a depth of 18 feet. The success of the investigation depends upon the speed and economy with which a large number of samples can be obtained.

For the purpose of pulling the soil tubes, which are driven into the ground with a 30-pound hammer, a new type of jack was designed and perfected.[2] This jack is light and simply made, and is extremely effective in its operation. The manner of its operation is shown in plate 1. Figure 1 shows the soil tube, the jack when first placed in position, the hammer used in driving the tube, and the grip. Figure 2 shows the grip as first placed around the tube. Figure 3 shows the grip as it has been tightened around the tube and the jack in position for raising the tube.

The hinged grip which fits around the soil tube has a conical outer face. With this, two rollers fitted on prongs of the lever arm are in contact when the jack is operated. Force applied downward on the handle moves the two rollers upward along the face of the cone, causing the grip to take hold and draw the soil tube out of the ground. When pressure on the handle is released, the springs at the end of the forks return the lever arm to its original position. At the same time, the grip slips down the tube of its own weight since the pressure on its conical face is released. The base of the fulcrum has an area of 120 square inches, giving ample bearing surface on the ground. For the sake of lightness the base is made of cast aluminum and the complete jack with handle and grip weighs less than 23 pounds.

When the apparatus is in its ordinary position, a force of 150 pounds applied 30 inches out on the handle gives a pulling effort of 1,800 pounds on the soil tube. This effort may be changed at will by applying the force on the handle at different positions or by moving the base so that the distance from the fulcrum of the point of contact of rollers and cone is varied.

Two of these jacks have been in operation for the past six months and have been put to severe tests, pulling tubes out of loose gravelly sand and also from

[1] Under the supervision of W. W. McLaughlin, Associate Chief of the Division of Agricultural Engineering, Bureau of Public Roads, United States Department of Agriculture.

[2] In coöperation with H. E. Twomley, designing draftsman of the Parker Machine Works, Riverside, California.

tight dry clay. In several cases, the pulling effort on the soil tube was estimated to be over 4,000 pounds. The jacks have pulled tubes wherever it was possible to drive them with 30-pound hammers. Jacks of this type cost $30 each.

PLATE 1

FIG. 1. FIRST POSITION OF JACK, HAMMER, AND GRIP
FIG. 2. GRIP AS FIRST PLACED AROUND THE TUBE
FIG. 3. JACK IN POSITION FOR RAISING TUBE

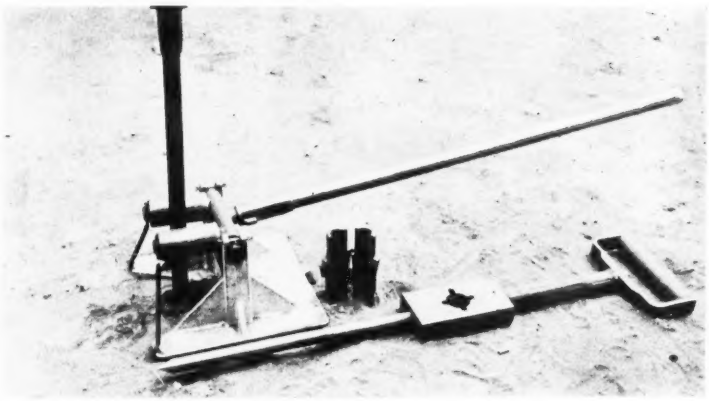

FIG. 1

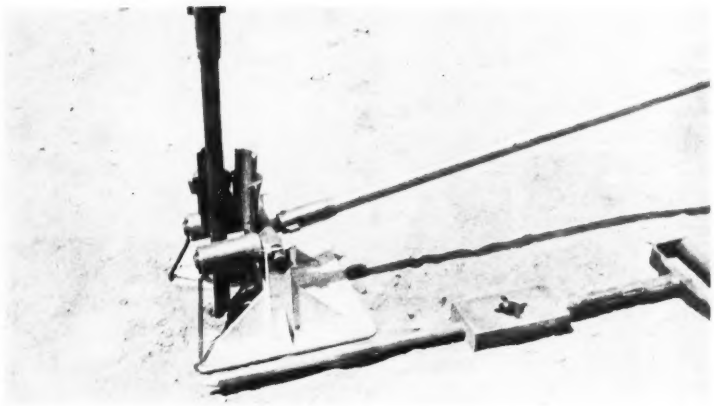

FIG. 2

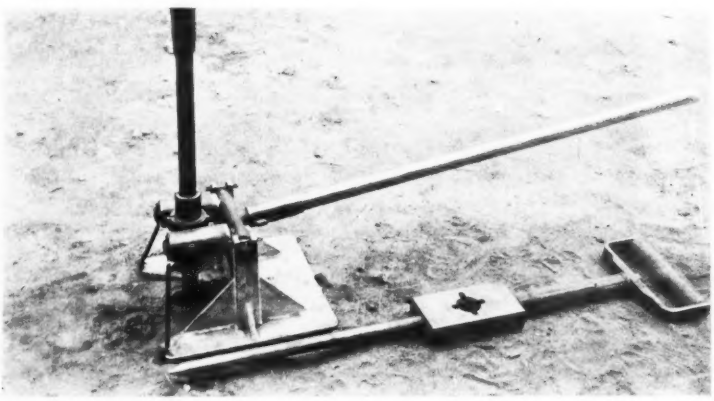

FIG. 3

SOME INFLUENCES OF THE DEVELOPMENT OF HIGHER PLANTS UPON THE MICROÖRGANISMS IN THE SOIL: II. INFLUENCE OF THE STAGE OF PLANT GROWTH UPON ABUNDANCE OF ORGANISMS

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The introductory material presented in the preceding report (2) demonstrates that development of plant roots leads to marked modification of the biological condition of the soil near the developing roots. This modification appears in the greater abundance of all groups of the microörganisms studied and also in the evolution of carbon dioxide from the soil. These results indicate merely that the modification of the soil population was apparent at the particular period of plant growth at which the studies were made. They give no idea as to whether the effects are the same throughout the growth of the plants or at what periods of plant growth the most marked effects may appear. Smith (1) observed that the growth of legumes increases the numbers of *B. radiobacter* beginning the third week and extending some weeks after harvesting the crop. Wilson and Lyon (3) grew pure cultures of organisms in soils in the presence and absence of plant growth. Although the results indicated that the numbers of bacteria are greater under plant growth, there was little to indicate that there is any consistent difference in the extent of the influence of the plants upon the organisms at different stages of plant development.[1]

The following studies were planned with the object of determining more conclusively the extent of the influences of different plants upon the soil population and particularly what differences might be exerted by the plants at different stages of growth.

EXPERIMENTAL PROCEDURE

The experimental studies were conducted upon soils in the greenhouse and in the field. The soil used for the greenhouse work was a fairly heavy acid soil (pH 5.5) being a natural mixture of Penn loam and Sassafras loam. This proved to be too heavy for best results. Although there was fairly good growth of most of the experimental plants, corn failed to make normal development. The plants used in the greenhouse studies were: oats, beans, rape, table beets, and sweet clover. Such plants were selected as would show a

[1] An extensive historical introduction to this subject may be found in part I of this series (2).

variety of characteristics of root development: fibrous roots, fleshy roots, tap roots, spreading roots, annuals, and biennials. There were 36 pots of soil involved in these studies, 6 planted to each of the five plants used and 6 left unplanted. Each pot contained 20 pounds of soil.

At each period of sampling, one pot of soil growing each of the plants and one unplanted pot of soil were used. The tops of the plants were harvested, the soil was then removed from the pots, the roots were separated as completely as possible from the soil, and then samples of the soil were passed through a 3 mm. sieve and brought to the laboratory for examination.

The legumes were inoculated at the time of planting. After all of the plants were well started they were thinned out to leave ten plants in each pot of oats, rape, and sweet clover; nine of beets; and six of beans.

The soils were kept close to the optimum moisture content throughout the experiment. Weeds were removed from the soil as they made their appearance. Since the soils were deficient in available nitrogen at the first period of sampling (36 days after planting) 2 gm. of sodium nitrate was added to each of the pots of soil except those supporting legumes.

The field studies were conducted upon a Sassafras loam soil of neutral reaction. The physical condition of this soil was good and brought about excellent development of all the plants which were used. The plants used in the greenhouse studies (oats, beans, rape, table beets, and sweet clover) were also used in the field and, in addition, potatoes, field corn, and mangel beets. Because the sweet clover was planted somewhat later than the other plants, differences will appear in the results in the recorded age of the sweet clover at the periods of sampling as compared with the other plants. The legumes were inoculated at the time of planting.

After the plants showed fair development they were thinned out sufficiently to prevent crowding. Throughout the season, weeds were eliminated by pulling by hand. One portion of the plot was left unplanted but otherwise treated identically as the planted soils. This unplanted section was used as a source of samples of soil supporting no plant development for comparison with samples of soil collected from about the roots of the various plants. In sampling the soils, the plants were carefully removed with some soil clinging to the roots. The soil was removed from the roots and samples were passed through a 3-mm. sieve and brought to the laboratory for study. Records were kept of the vigor and stage of growth of the plants as well as of the weights of the tops and root systems at each period of sampling.

The soils collected from the unplanted section and from the roots of the plants were examined for the abundance of filamentous fungi, actinomyces, bacteria developing upon albumin agar, organisms developing upon nitrogen-free mannite agar, and mucoid colonies (*B. radiobacter*) developing upon nitrogen-free mannite agar as outlined in the preceding report (2). The abundance of *B. radiobacter* was also determined by plating the soils upon glycerol-nitrate-soil extract agar as suggested by Smith (1). This method was slightly modified

in that just before pouring the plates, the crystal violet was added to the melted agar (in 300 cc. portions in 500 cc. Erlenmeyer flasks) in such amounts as to create a concentration of 1 part of dye to 100,000 parts of medium. Smith added the dye to the soil suspensions before adding them to the plates. An attempt was also made to determine the abundance of nitrogen fixing organisms. This involved the introduction of 1-cc. portions of dilutions of the soils

TABLE 1

Influence of development of higher plants upon abundance of bacteria (1/1,000,000)

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	34.2	14.6	30.4	19.8	17.0	23.2
Oats	33.6	42.0	88.0	34.6	29.2	45.5
Corn	40.4	29.8	42.6	48.0	37.0	39.6
Beans	26.7	26.0	47.8	51.8	42.8	39.0
Potatoes	36.0	23.4	38.0	21.3	14.8	26.7
Table beets	37.2	22.6	60.4	41.4	47.8	41.9
Mangel beets	44.7	38.4	48.0	48.6	51.8	46.3
Rape	71.2	46.8	43.6	69.4	88.4	63.9
Sweet clover*	34.8	27.2	29.2	33.0	50.2	34.9

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	28.3	34.6	19.3	21.4	17.4	9.0	21.7
Oats	30.8	20.5	31.2	50.0	36.2	31.4	33.4
Beans	36.6	45.3	22.0	28.2	15.8	12.4	26.7
Beets	26.5	57.5	19.8	31.7	42.0	13.6	31.9
Rape	21.8	93.7	46.5	157.6	24.6	19.6	60.6
Sweet clover	35.4	36.8	36.0	21.8	29.0	9.8	28.1

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

in duplicate into flasks containing 100-cc. portions of nitrogen-free dextrose solutions. Similar determinations conducted in mannite solutions indicated the presence of much smaller numbers than did the dextrose medium. Records were kept of the dilutions which resulted in fixation of nitrogen and those from which no fixation occurred after 30 days.

EXPERIMENTAL RESULTS

Numbers of bacteria

Table 1 and figure 1 indicate the results of the enumeration of bacteria. In the table the results of both the field and greenhouse study are included;

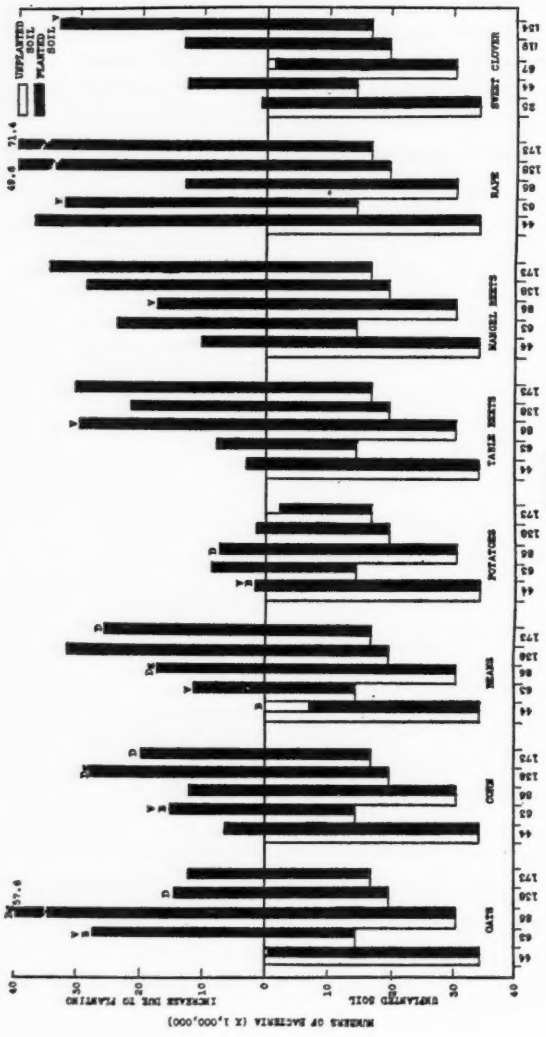

FIG. 1. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF BACTERIA IN THE SOIL

only the field results are presented graphically since the two sets of data are much the same. The plant growth was so much more vigorous and normal in the field that the results from studies of these plants should be much more characteristic than those of the greenhouse plants. In the greenhouse all of the plants except the oats became quite stunted during the last periods of the extended study. It is also apparent that, in soils kept for long intervals, soluble salts may accumulate to concentrations far greater than would occur in the field in humid regions.

In the figure, the unshaded columns extending below the zero line represent the abundance of bacteria in the fallow soil. The black columns represent the abundance of bacteria in the soil obtained from the rhizosphere of the various plants. Since the black columns extend below the zero line to the point of the unshaded columns any extension of the black columns above the zero line represents the detection of greater numbers of bacteria in the planted soil. Likewise, if the black columns do not extent to the zero line it indicates that the numbers found in the planted soil were smaller than those found in the unplanted soil.

Since most of the black columns extend above the zero line it is apparent that the development of higher plants has in practically all cases increased the abundance of bacteria which are detected by this plate method. In some cases the increases are much more pronounced than in other instances. More than this, there are certain differences which may be ascribed to the difference in type of development of the various plants and, further, some of these variations may be correlated with the stages of growth of any one plant. It is to be expected that plants showing such a variety of growth characteristics (fibrous and fleshy roots, tubers, annuals, biennials, legumes, and non-legumes) would affect the soil population differently. In general, these results show that the influences of most of the plants are not great in the early stages of plant growth and that the maximum effects generally appear only after the plants have attained considerable size, have reached the height of vegetative growth, bloomed, or started vegetative degeneration. Subsequent to the death of the plants there is generally a pronounced decline in the abundance of bacteria.

The letters written above the columns on the figure indicate the stages of plant development at the different periods of sampling the soils (*B*—blooming, *V*—height of vegetative development, *Dg*—initial degeneration, *D*—death).

Under the potatoes which mature and die within a short period of time there is a marked increase in numbers after the early stage of growth and pronounced decline subsequent to maturity and death of the plants. A similar striking change is observed under the oats. With the corn and beans the decline after death is not as apparent since the plants were killed by frost just before the last sampling and the interval between death and sampling was too short for the biological activities to drop to a low level. The biennials show a very marked effect upon the organisms even at the last periods of sampling. At this time these plants were in active development while the annuals were all

dead. This difference between the annuals and biennials does not appear to be as striking with the greenhouse soils as with the field soils, presumably because of the degenerated condition of all the plants in the greenhouse at the late periods.

This periodic influence of the plants may logically be considered as being correlated with the periods of youthful root development accompanied by little

TABLE 2

Influence of development of higher plants upon abundance of organisms developing upon nitrogen-free mannite agar (1/1,000,000)

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	33.3	26.2	17.8	8.0	12.2	19.5
Oats	28.6	44.2	52.6	13.6	15.0	30.8
Corn	39.0	34.8	34.6	23.2	21.0	30.5
Beans	39.8	31.2	33.6	25.4	30.4	32.1
Potatoes	31.0	42.8	29.8	13.0	15.8	26.5
Table beets	45.4	31.5	49.0	19.8	32.2	35.6
Mangel beets	65.8	38.8	36.6	22.8	34.4	39.7
Rape	81.2	70.8	46.8	18.6	45.4	52.6
Sweet clover*	35.7	33.4	26.0	16.4	23.2	26.9

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	13.8	14.0	6.4	5.0	9.4	11.0	9.9
Oats	21.0	14.0	19.4	28.4	23.6	30.2	22.8
Beans	29.8	30.0	12.8	19.4	12.4	14.3	19.8
Beets	16.3	21.2	9.2	27.4	21.4	23.3	19.8
Rape	20.0	49.8	19.0	105.4	15.6	23.2	72.2
Sweet clover	26.6	32.2	17.6	21.0	17.4	16.6	21.6

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

root excretion of microbial foods, of periods of active root excretion of considerable amounts of material available to the microörganisms as food, and finally of root degeneration and death with only the more resistant materials becoming available to the microörganisms with no further replacement of these substances by the plant.

It may be noted that there appears to be a general tendency for the bacteria in the unplanted soils to decrease throughout the period of study. This might be expected since, in a fallow soil, the readily decomposable organic

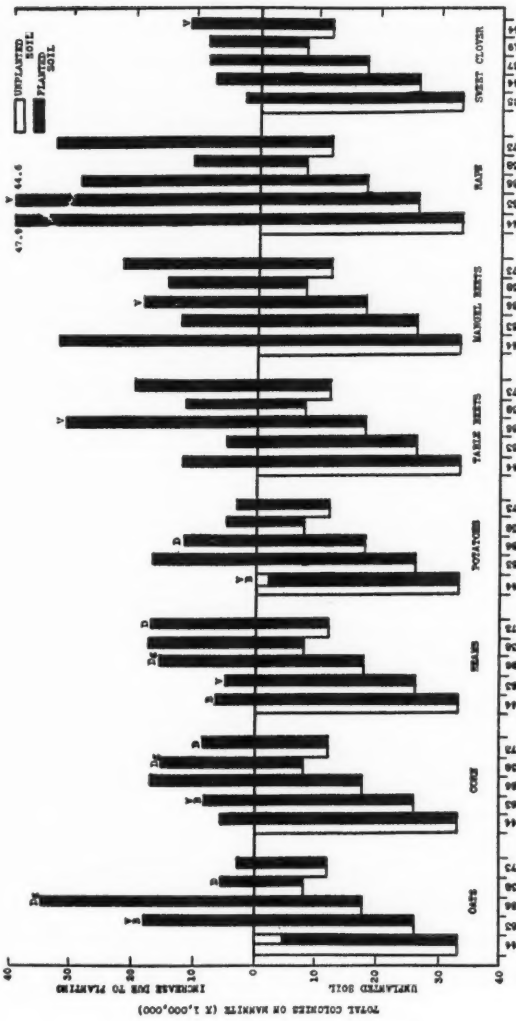

FIG. 2. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL WHICH GROW UPON NITROGEN-FREE MANNITE AGAR

matter is becoming continuously depleted and microbial foods are consequently less abundant. The marked effect of rape upon the bacteria is particularly striking and appears also in the studies of the other groups of organisms, as will be seen in the following pages. It is equally apparent that there is little correlation between the sizes of the plants and their effects upon the bacteria since the oats exerted greater effects than the field corn, and the beans produced

TABLE 3

Influence of development of higher plants upon abundance of B. radiobacter *group developing upon nitrogen-free mannite agar (1/100,000)*

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	9.8	9.4	3.8	1.0	3.4	5.5
Oats	6.6	31.4	19.2	2.6	7.0	13.4
Corn	12.8	18.4	8.0	3.8	8.6	10.3
Beans	13.6	15.0	6.8	9.0	14.0	11.7
Potatoes	11.2	11.4	5.2	2.2	5.8	7.2
Table beets	10.2	10.4	12.0	3.2	10.6	9.3
Mangel beets	20.8	17.2	9.0	7.8	14.8	13.9
Rape	31.8	27.0	11.6	8.4	12.0	18.2
Sweet clover*	7.2	16.6	7.0	3.6	12.4	9.4

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	8.8	5.4	3.4	1.4	0.6	2.4	3.7
Oats	16.2	10.5	16.0	9.8	16.0	21.2	15.0
Beans	21.0	19.6	16.5	10.2	5.0	10.0	13.7
Beets	8.6	12.3	6.4	8.3	8.0	7.0	8.4
Rape	15.4	20.0	17.3	10.0	9.3	9.4	13.6
Sweet clover	17.4	16.5	12.0	10.8	10.2	5.5	12.1

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

effects comparable to the corn. These three plants differed very greatly in amounts of root systems, vegetative growth, and seed development.

Colonies on mannite

The influences of plant development upon the abundance of organisms which grow on nitrogen-free mannite agar are shown in table 2 and figure 2. The organisms developing upon this medium are much the same as those appearing upon the albumin agar and, as might be expected, they manifest much

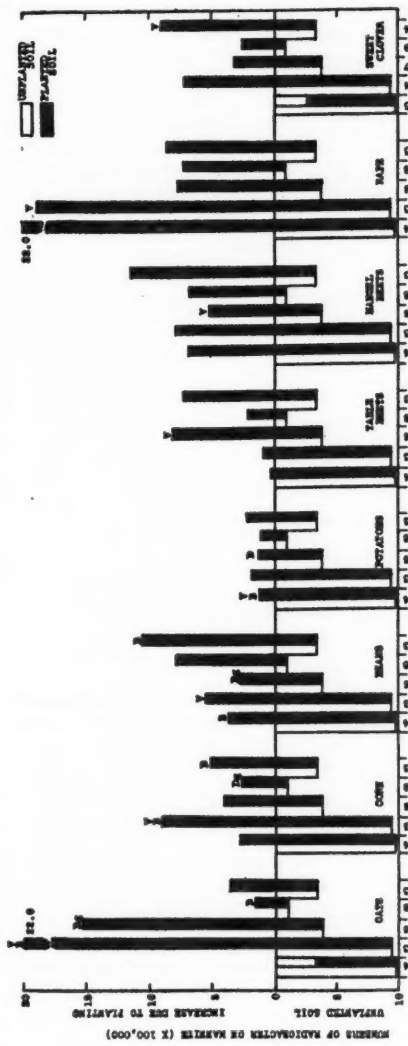

FIG. 3. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL OF THE *B. radiobacter* GROUP WHICH GROW UPON NITROGEN-FREE MANNITE AGAR

the same responses to plant development. These responses appear in the relatively low numbers of organisms at early stages of plant growth (except with the mangel beets and rape), maximum numbers in most cases at advanced periods of plant growth, depression in numbers subsequent to death of the plants, relatively high numbers at advanced periods in growth of biennials as compared with annuals and, in all observations, much greater numbers of or-

TABLE 4

Influence of development of higher plants upon abundance of B. radiobacter *group developing upon glycerol-nitrate agar (1/10,000)*

Field soils

PLANT	AGE OF DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	5.4	9.2	9.0	3.2	4.2	6.2
Oats	7.8	78.0	63.2	8.6	6.7	32.9
Corn	6.8	20.2	31.8	33.4	9.6	20.4
Beans	19.8	25.4	44.0	3.6	16.4	21.8
Potatoes	7.8	9.8	53.4	12.0	5.0	17.6
Table beets	8.4	15.4	35.6	21.8	13.8	19.0
Mangel beets	14.0	64.0	31.6	40.0	14.0	32.7
Rape	466.0	86.0	63.6	51.2	36.4	140.6
Sweet clover*	11.4	20.0	19.0	6.2	28.2	17.0

Greenhouse soils

PLANT	AGE OF DEVELOPMENT					AVERAGE OF ALL PERIODS
	59 days	93 days	128 days	169 days	202 days	
Fallow	15.0	9.0	1.2	1.0	0.4	5.3
Oats	6.0	13.3	6.2	48.2	18.8	18.5
Beans	36.6	20.6	51.2	9.2	8.4	25.2
Beets	13.8	8.6	10.8	2.8	8.4	8.9
Rape	31.0	22.4	27.4	2.6	3.2	17.3
Sweet clover	17.4	13.6	24.6	18.4	4.6	15.7

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

ganisms in soils near plant roots as compared with the abundance in unplanted soils. As with the bacterial counts, the numbers of the organisms show a progressive decrease in the unplanted soil as time elapses. There is a striking difference in the influences of the different plants upon the organisms; the most marked effect appears under the rape and the most transitory effect under potatoes. The explanation for these apparently characteristic effects of different plants still remains to be determined.

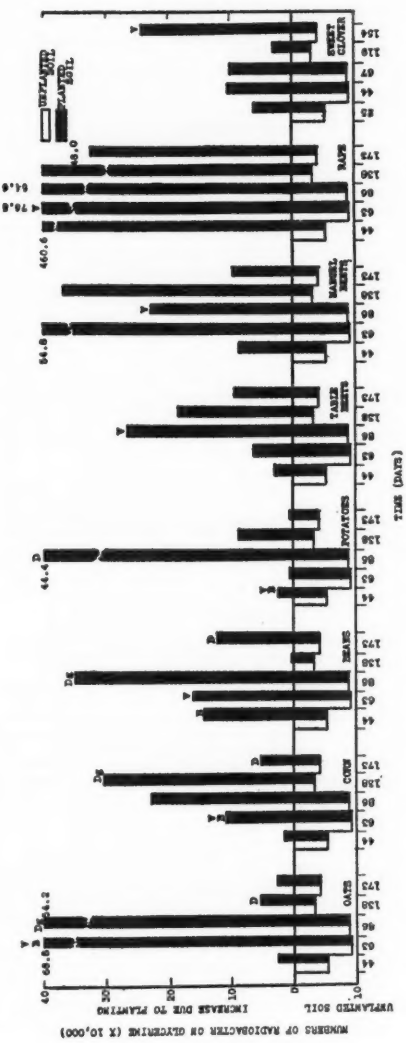

FIG. 4. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL OF THE *B. radiobacter* GROUP WHICH GROW UPON GLYCEROL AGAR

B. radiobacter group developing upon mannite

As seen by table 3 and figure 3, the organisms producing mucoid colonies upon nitrogen-free mannite agar (*B. radiobacter group*) also increased about the plant roots. In fact the proportional increases are somewhat greater than appeared in the organisms previously considered. The oats and rape showed the most pronounced increase. The potatoes showed comparatively slight influence upon this group of organisms at any stage of growth. The

TABLE 5

Influence of development of higher plants upon abundance of actinomyces (1/1,000,000)

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	12.8	7.0	12.0	7.4	10.2	9.9
Oats	10.8	6.4	10.0	10.6	10.8	9.7
Corn	11.6	7.6	11.0	13.0	8.0	10.2
Beans	14.3	8.2	15.4	11.0	10.4	11.9
Potatoes	9.7	10.0	11.2	14.0	10.0	11.0
Table beets	10.3	9.0	17.0	12.3	14.2	12.6
Mangel beets	19.0	9.0	16.2	11.2	17.2	14.5
Rape	12.0	11.5	9.2	9.8	10.8	10.7
Sweet clover*	10.0	10.0	13.3	8.0	12.0	10.7

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	5.5	7.0	4.5	5.8	3.0	3.8	4.9
Oats	4.6	3.8	3.6	6.4	4.8	7.2	5.1
Beans	6.0	8.3	5.0	6.8	5.8	5.4	6.2
Beets	5.0	7.5	4.8	4.8	4.2	4.6	5.2
Rape	5.0	16.8	7.5	11.6	4.8	5.4	8.5
Sweet clover	7.4	7.0	8.0	7.6	3.8	4.0	6.3

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

beans showed an unusually pronounced effect upon these organisms at the final stages of growth. In general, the course of the changes in the numbers of these organisms in both the planted and fallow soils appear much the same as in the organisms previously considered.

B. radiobacter group developing upon glycerol

The results concerned with this group appear in table 4 and figure 4. Pronounced increases in the abundance of these bacteria appeared under the

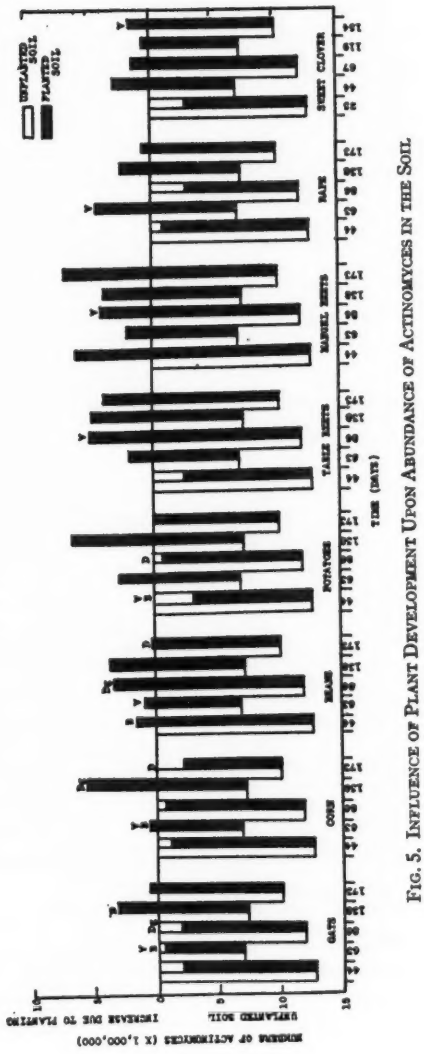

FIG. 5. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF ACTINOMYCES IN THE SOIL

developing plants. The proportional increases are more pronounced than those observed with any of the other groups of organisms which were studied. Here the influence of rape is particularly great. The organisms from soil growing this plant reached the point of 4.6 millions, while the unplanted soil at the same period showed the presence of only 54 thousands. However, the absolute increases in most cases are much lower than with the other organisms previously considered since this group of bacteria makes up a much smaller portion of the

TABLE 6

Influence of the development of higher plants upon abundance of filamentous fungi (1/10,000)

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Fallow	16.8	9.0	13.0	27.0	23.2	17.8
Oats	18.2	21.6	26.8	21.0	19.4	21.4
Corn	14.0	18.6	16.0	28.0	28.0	20.9
Beans	24.6	16.0	23.8	22.8	31.4	23.7
Potatoes	24.8	13.6	17.6	29.0	19.4	20.9
Table beets	11.8	15.3	22.6	18.8	25.4	18.8
Mangel beets	23.8	18.5	27.8	18.6	26.6	23.1
Rape	14.4	16.3	12.2	28.4	28.6	20.0
Sweet clover*	16.0	16.2	16.4	16.2	21.2	17.2

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Fallow	19.6	21.2	14.0	23.0	16.6	16.2	18.4
Oats	25.8	13.8	20.0	20.0	12.2	22.6	19.1
Beans	18.8	23.2	19.2	19.0	16.0	10.4	17.8
Beets	20.2	17.8	21.8	26.2	21.6	21.8	21.6
Rape	20.6	28.6	17.2	18.6	18.4	13.4	19.6
Sweet clover	21.0	19.0	20.6	14.0	22.8	14.2	18.6

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

total soil population than the other groups. In view of the pronounced response of these bacteria to plant growth it would be of considerable interest to ascertain what factors are responsible for this change.

The qualitative differences between the influences of the annuals and biennials are less pronounced upon these organisms than upon the general bacterial population of the soil. With the exception of the soil supporting sweet clover the numbers of the *B. radiobacter* group decreased at the late periods of plant development in all cases.

Numbers of actinomyces

The results of the observations of the actinomyces presented in table 5 and figure 5 show that this group of organisms is affected by plant development very differently than are the other groups of organisms previously studied. The increases are generally erratic and show little or no correlation with the stages of plant development. Likewise their abundance in the unplanted soil shows little definite change during the season. If they are influenced by the plant development, the method used for their study has not detected the effect. At the most, it might be conceded that the development of plants has increased the abundance of actinomyces about the root systems but that this increase is comparatively slight. Of 70 cases observed (40 from field soils and 30 from greenhouse soils), 50 showed greater numbers of actinomyces in the planted soils than occurred in unplanted soils, while 19 showed fewer actinomyces in the planted soils.

Numbers of filamentous fungi

The group of filamentous fungi show as little variation in numbers which may be interpreted as response to plant growth as do the actinomyces. These results are presented in table 6 and figure 6. The numbers generally appear to be somewhat greater at the stages of extensive growth and are quite low in soils supporting annuals subsequent to degeneration of the plants. However, it is difficult to explain the low level of fungus numbers at the late stages of development of the biennials in case one accepts as a fact that such a method of studying the fungi reflects the abundance of their occurrence in the soil.

In the light of our present understanding of the activity of filamentous fungi in soils it seems logical to conclude that these results do not properly indicate the extent of influence of plant development upon this group of organisms. Although it appears that the fungi were more abundant near the plant roots, the method of determination is so unsatisfactory that, unless pronounced differences are obtained, it seems unwarranted to interpret the differences as being more than suggestive. In the 70 cases studied, 45 showed greater numbers of fungi about plant roots and 25 showed greater numbers away from the roots. The unplanted soils showed no consistent change in numbers of fungi during the period of study.

Numbers of organisms fixing nitrogen

As outlined with the experimental methods these organisms were estimated by inoculating diluted suspensions of soil into flasks of nitrogen-free nutrient solutions. As each period of sampling, the suspensions were inoculated into flasks in duplicate at each of five dilutions including the limits of growth of the organisms. It frequently occurred that organisms would develop at certain high dilutions of the series with no growth at some of the lower dilutions, causing "skips" in the series. With only two cultures at each dilution and so many "skips" in the series it is not possible to determine the number of nitrogen-

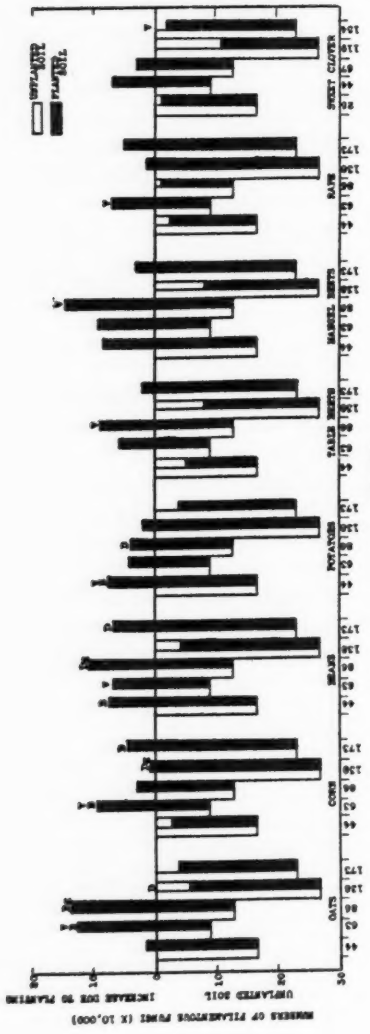

FIG. 6. INFLUENCE OF PLANT DEVELOPMENT UPON ABUNDANCE OF FILAMENTOUS FUNGI IN THE SOIL

fixing organisms with any degree of accuracy. In order to suggest the general trend of the results, however, a summary is presented in table 7. These data present merely a list of the number of cultures in each series at each dilution which showed fixation of nitrogen. In the series of studies of the greenhouse soils a mannite medium was used but this failed to support development of nitrogen-fixing organisms at dilutions of the soil greater than 1,000. With the

TABLE 7

Numbers of cultures showing nitrogen fixation from planted and unplanted soils

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE OF ALL PERIODS
	44 days	63 days	86 days	138 days	173 days	
Cultures used	10	10	10	10	10	10
Fallow	6	6	7	3	5	5.4
Oats	8	8	7	4	3	6.0
Corn	8	6	6	5	7	6.4
Beans	8	5	6	5	6	6.0
Potatoes	6	7	5	4	5	5.4
Table beets	8	8	4	7	5	6.4
Mangel beets	8	7	7	7	7	7.2
Rape	8	5	5	6	5	5.8
Sweet clover*	8	6	7	3	5	5.8

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT						AVERAGE OF ALL PERIODS
	36 days	59 days	93 days	128 days	169 days	202 days	
Cultures used	10	10	10	10	10	10	10
Fallow	4	3	4	5	5	6	4.5
Oats	3	2	9	7	5	8	5.7
Beans	3	2	5	5	5	6	4.3
Beets	4	4	6	7	7	6	5.7
Rape	2	3	4	7	7	9	5.3
Sweet clover	3	2	7	9	3	7	5.2

* For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

field soils a dextrose medium was used and nitrogen fixation occurred quite regularly at dilutions of the soils of hundreds of thousands and frequently of millions. It is quite apparent that, irrespective of the medium used, the type of plant developing upon the soil, or the stage of development of the plants, there is nothing which appears sufficiently different from the results from unplanted soils to suggest that plant growth appreciably modified the abundance of nitrogen-fixing organisms in the soil. Although these organisms appeared

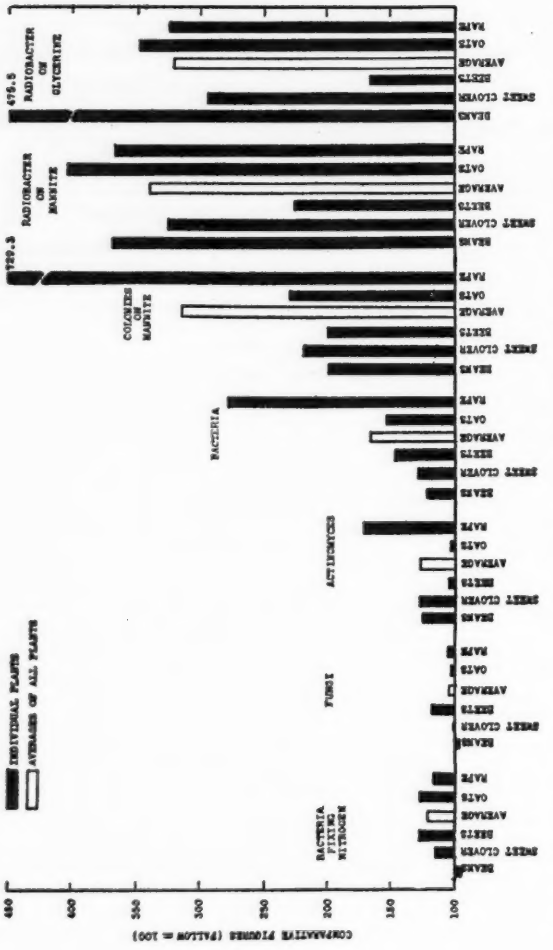

FIG. 7. AVERAGES OF THE GREENHOUSE STUDIES OF THE INFLUENCES OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL

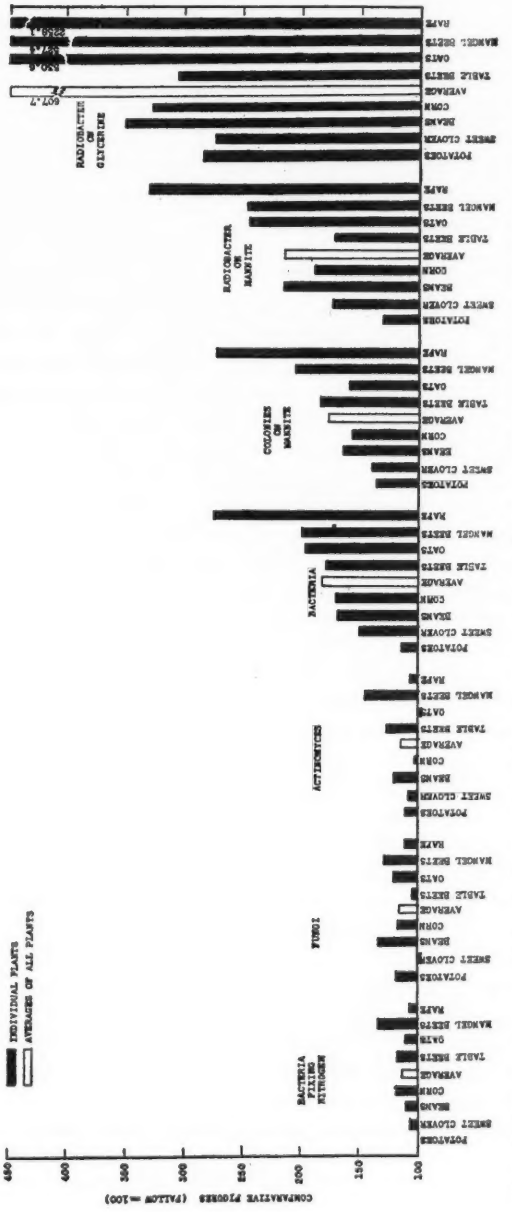

FIG. 8. AVERAGES OF THE FIELD STUDIES OF THE INFLUENCES OF PLANT DEVELOPMENT UPON ABUNDANCE OF ORGANISMS IN THE SOIL

to the extent of hundreds of thousands no consistently greater number of these bacteria developed from soils obtained from the rhizosphere than from soil obtained from a distance from the roots. Further, the legumes showed no more effect upon these organisms than did the non-legumes. In view of the plausable theoretical possibility that the rhizosphere may be a suitable habitat for development of nitrogen-fixing bacteria it appears strange that no results were obtained which would verify this supposition. The possibility still remains that the method which was used was unsuited to determine the abundance of this group of organisms.

Averages of influences of plants upon all the groups of organisms

In figures 7 and 8 the data are presented as averages of the determinations at all periods of sampling. Comparative figures are given, the results from the unplanted soil being considered as 100. The unshaded columns are averages of the average effects of all the plants upon each of the groups of organisms. In each group, the data are arranged from left to right in the order in which the different plants affect the abundance of bacteria in the soil.

It is evident that plant development exerts very different proportional effects upon different elements of the soil population. The apparent effects upon the nitrogen-fixing organisms and upon the fungi and actinomyces are very slight. The bacterial population as a whole and the organisms developing upon mannite agar are affected to a marked degree and in much the same order with certain few exceptions. The bacteria of the Radiobacter group increased to a much greater proportional degree than any of the other organisms. This is particularly striking with the results of their detection upon glycerol media from the field soils. Among all of the data, the striking influence of rape upon all the groups of soil organisms is clearly apparent. It was expected that the legumes would effect a somewhat more marked influence upon the soil population than is apparent from these results in view of the fact that it has been repeatedly claimed that such plants have the most pronounced influence upon soils. Since, at all the periods of study, the sweet clover plants were less mature than any of the others the comparison of their effects during early stages of growth does not accurately indicate their influence upon the soil organisms over extended periods of time.

If the order of the influence of the plants upon the numbers of bacteria in the soil during the growing season was the same for all of the biological groups, it might be assumed that this order of the plants represented the order of their influences upon the microbial population as a whole. In some cases this appears to be the case, but it appears more logical in many cases to assume that the order of influence of a group of plants upon one group of soil organisms may not be the same upon all soil organisms. These influences of different plants may be so markedly altered by cultural conditions which modify the extent of plant growth, the period of time before maturity, and other physiological characteristics of plants that any attempt to indicate without qualifi-

cation what plants exert the greatest or slightest effects upon the soil organisms would be replete with errors. It is more desirable to ascertain what factors associated with plant growth are responsible for this effect upon the soil organisms.

TABLE 8

Correlation between weights of roots of single plants at the periods of sampling the soils and the influences of the plants upon the abundance of bacteria in the soil

Field soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE INCREASE IN NUMBERS OF BACTERIA DUE TO PLANT GROWTH
	44 days	63 days	86 days	138 days	173 days	
						millions
Potatoes	3.36	8.91	25.1*	28.3†	51.0†	3.5
Sweet clover‡	0.003	0.08	0.6	10.5	61.0	11.7
Beans	0.47	0.66	0.7	2.1	1.2	15.8
Corn	3.36	12.26	38.0	48.0	41.0	16.4
Table beets	0.35	5.8	21.8	28.5	22.5	18.7
Oats	0.25	0.31	0.3	0	0	22.3
Mangel beets	0.34	7.1	26.5	90.3	77.3	23.1
Rape	0.99	1.20	2.0	6.8	7.7	40.7

Greenhouse soils

PLANT	AGE OF PLANT DEVELOPMENT					AVERAGE INCREASE IN NUMBERS OF BACTERIA DUE TO PLANT GROWTH
	59 days	93 days	128 days	169 days	202 days	
						millions
Beans	0.136	0.068	0.085	0.131	0.069	5.0
Sweet clover	0.002	0.006	0.247	0.252	0.314	6.4
Beets	0.008	0.071	0.298	0.511	0.315	10.2
Oats	0.024	0.080	0.304	0.341	0.124	11.7
Rape	0.015	0.073	0.414	0.541	0.420	38.9

* Mainly tubers only 0.56 gram fibrous roots.

† All tubers, no fibrous roots.

‡ For sweet clover the sampling periods are 25, 44, 67, 119, and 154 days.

Influence of extent of root systems

It might be expected, since it is through the root systems of the higher plants that the influences become exerted upon the soil organisms, that there would be some correlation between the abundance of the roots and the extent of the changes in the organisms in the soil. An inspection of table 8 indicates quite definitely that there is little or no correlation between the weights of the plant roots and the average increases in bacteria in soils under the plants. There is no greater correlation between the weights of roots at any one period of

sampling and the abundance of bacteria under the plants at that period. Further, no apparent correlation exists between the root weight and the abundance of any of the other organisms which were studied.

It may not seem justified to compare the weights of roots of single plants since different numbers of plants covered equal areas of soil. It would seem unlikely, however, that any comparison of weights of roots occupying equal volumes of soil would show correlated relationships between the effects of these roots and the soil population. It might be logical to assume that correlations would exist between the extent of root surfaces and the influences of the plants in any unit volume of soil. It seems more likely that none of these factors would have more than a chance relationship with the observed effects. Other factors related to the physiological behavior of the plants undoubtedly control the reactions. It seems apparent that young roots exert comparatively slight effects and that, at the period close to fruiting or at advanced stages of growth, the plants exert the most marked influences. This would appear to suggest that factors associated with the quality and quantity of root excretions (particularly organic) might be factors of major importance in this connection. These are related to certain characteristics inherent in the plants themselves and would not justify an assumption that the abundance of root systems would be of most importance in determining the observed changes.

DISCUSSION

It is quite apparent that the plate method of determining the abundance of organisms in the soil is far from satisfactory. However, the results which have been obtained appear of such an order as to indicate that the apparent effects of the plants upon the soil organisms are significant effects in most cases. The fact that marked effects of plant growth upon the soil organisms were observed by the methods which were used may suggest that even more marked effects are actually brought about by the plants. This would seem likely since the large samples of soil which were taken for analysis necessitated the collection of considerable soil which was not in contact with the plant roots. If it is assumed that root excretions are of major importance in determining the results, the greatest effects should appear closest to the roots. Further studies of more detailed nature of conditions in the zone of the root surfaces should indicate that the microörganisms are even more profoundly modified by plant growth than the present results would suggest.

It is recognized that the periods of testing the soils were so infrequent that many significant changes in the soil population may have been overlooked. The points of maximum effects and of inflection would undoubtedly have been more accurately located by more frequent observations of the soils. It is apparent from the data which are available, however, that certain pronounced differences are expressed in the abundance of the organisms at different stages of plant growth.

In view of the present observations it would seem that plant growth is one of the most important factors in determining the irregular distribution of microorganisms in soils. Samples of soil from an apparently uniform field may vary greatly in abundance of organisms if they are gathered near or away from roots or if they contain a small or large amount of roots. The influences of the plants vary so greatly during the course of root development that observations from a single period would not be representative of other periods. In this connection, variations in the influences of the plants upon the organisms at different stages of growth may be of importance in determining the so-called seasonal fluctuation of microörganisms in soil, where some of the variations appear to be related to neither the moisture nor temperature. At least, it may be concluded that samples of soil gathered about different plants at any one period give no true representation of the comparative influences of the different plants upon the soil population. Any comparison which is to represent the facts properly must indicate the course of the influences throughout the growth of the plants.

The fact that plant growth favors the appearance of larger numbers of organisms in the soil, at least at certain stages in plant development, indicates why, under certain conditions one may expect to find some close correlations between crop yields of soils and abundance of microörganisms in the soils. Provided that the soils concerned in the comparison were originally alike and that the same crop had been continuously planted on these soils, one would expect that, where the greatest plant development occurred, there would be the greatest amount of root residues and that the other effects associated with the influences upon the organisms would be such as to exert the most profound effects upon the soil population. On the other hand, knowing that different plants affect the soil population differently at any stage in their growth it would hardly be expected that even similar soils supporting different plants under different fertilizer treatments would always show correlations between yields of plants and abundance of microörganisms in the soils. This relationship is further complicated where soils essentially different in physical and chemical condition are concerned. Even though crop yield should be high from a soil which was normally of low biological activity the abundance of organisms might not be so great as from a soil naturally of a high level of biological activity. At the same time the actual increase in abundance of organisms resulting from plant development might be the same in both soils. The growth of crops in rotations further complicates an interpretation of the final results upon soil organisms.

SUMMARY

Results are reported of studies concerned with the influences of the development of higher plants upon the abundance of microörganisms in the soil. A considerable variety of different plants was cultivated in the greenhouse and field and, during their growth, observations were made periodically upon the

abundance of bacteria, actinomyces, filamentous fungi, organisms developing upon nitrogen-free mannite agar, organisms of the *B. radiobacter* group developing upon nitrogen-free mannite agar, organisms of the *B. radiobacter* group developing upon glycerol-nitrate agar, and nitrogen-fixing organisms developing in nitrogen-free dextrose or mannite solutions. The following conclusions appear to be justified as a result of these studies:

1. The development of higher plants exerts pronounced influences upon the soil population but these influences are greater upon some organisms than upon others. The proportional increases in nitrogen-fixing organisms, actinomyces, and filamentous fungi are slight. The greatest proportional increases appear in the *B. radiobacter* group of organisms although very striking effects are apparent in the general bacterial population. The average of the seasonal effects of different plants upon the different bacteria varied between increases of 15 per cent and several hundred per cent.

2. Different plants exert different degrees of influence upon the soil organisms; some, as potatoes, consistently increased the numbers slightly, while others, as rape, increased the numbers to a striking degree in most cases.

3. The extent of the influence of any one plant upon the soil population is different at different stages of growth. Slight effects are apparent in early stages of growth, maximum effects appear only after the plants have reached considerable size, and the influences become less pronounced subsequent to the death of the plants. Consequently, the length of the growing period is an important factor in determining the degree of the effect of plants upon microörganisms. Because of the longer growing periods of biennials these plants show a much more prolonged effect upon the organisms than do annuals. Legumes may exert no more pronounced effects upon soil organisms than do non-legumes.

4. The extent of the effects of plants upon the soil organisms is not determined by the size of the different plants or the extent of root development but may be associated with some characteristics of the physiology of the plants, particularly as regards quality and quantity of root excretions. Many factors are undoubtedly concerned in these changes.

5. The results emphasize the fact that higher plants are of great importance in bringing about an unequal distribution of microörganisms in the soil and may be a major factor in determining the so-called seasonal fluctuation of microörganisms in soil where temperature and moisture do not appear to be related.

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THE BOTANICAL COMPOSITION AND MORPHOLOGICAL FEATURES OF "HIGHMOOR" PEAT PROFILES IN MAINE[1]

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During recent years the study of peatlands and their differentiation has tended more and more to emphasize the importance of rather detailed and varied knowledge of profile features. It has come to be regarded a necessary part of any successful attempt in bringing peat soils into agricultural or industrial use, to approach the examination of peat profiles upon the basis of the botanical composition and morphological characteristics of the several layers and "horizons" found at different depths from the surface to the mineral subsoil. Likewise an increasing amount of attention is given the effort to characterize more completely any type of peat area with all its properties and peculiarities, that is, to determine accurately the several outstanding physical properties, the chemical composition of various organic complexes, and the microbiological population of peat profiles under virgin conditions, in order to contribute to an understanding of the changes that take place in similar areas of peat under conditions of cultivation and lowered water table.

Most of the criteria by which we determine the outward characteristics and geographical mode of origin of peatlands, and analyze their different profile features are relatively well known and readily applied. But the problem becomes involved and often very complex when we attempt to coördinate the results of the various analyses, or when we seek to establish the probable degrees or stages of decomposition from the initial, virgin peat profile to the fully developed mature profile, to arrange the changes in a proper sequence, to determine the rate of decomposition and time involved, as well as their relationship to any given environment in which the changes occur.

The result of focussing the efforts of modern peat investigations upon the examination and analysis of the profile features of three raised bogs or highmoors in Maine furnishes the theme for the following two related studies. This paper constitutes an endeavor to give the botanical and morphological profile record reflecting the succession of former vegetation units now forming peat deposits, whereas the paper by Waksman and Stevens, published elsewhere in this issue of the journal, emphasizes certain chemical and bacterio-

[1] A detailed analysis of the chemical composition and the bacteriological activities in the various layers of these profiles is published elsewhere in this issue of the journal, by Waksman and Stevens.

logical methods as the approach to the complex problem of establishing relationships between different types of peat (5). A discussion of the physical properties and the coördination of analytical studies with regional profile differences is contemplated in a later paper.

The field work was carried on in coöperation with S. A. Waksman from August 6 to August 15, 1928, and afforded not only the opportunity of continuing earlier studies on New England peat profiles (1) but also of obtaining fresh material, especially from layers of peat at considerable depth below the surface.

The localities chosen were the Denbo heath near Cherryfield, the Veazie heath southwest of Orono, and the Lewiston or Garcelon heath at Lewiston, Maine. Aside from the matter of accessibility these areas of peat are better known than others, and further experimental work can be carried on with the use of the facilities of the university and agricultural experiment station at Orono as a base.[2]

METHODS OF PROCEDURE

The study of the peat profiles, the botanical identification of the successive layers of plant remains, and the separate morphological features were carried out as follows:

Peat samples from the surface to 3 feet, intended for obtaining analytical data from the standpoint of volume weight and for determining the changes in certain physical properties, were collected by means of a new type of sampler. The instrument was made by C. J. Crawley in the mechanical laboratory of this Bureau. It consists of a duraluminum or brass tube approximately 1½ inches (3.75 cm.) in diameter and 10 inches (25 cm.) long. At one end is a detachable device 2 inches (5 cm.) long provided with a slit about 1¾ inches (4.5 cm.) from its cutting edge. The sampler is inserted without turning into the walls of the freshly dug hole. The peat material in the outer, projecting edge of the tube is cut off with a spatula through the slit-like opening in the tube. The surplus material from the inner, inserted end of the tube is cut off with a sharp knife even with the cutting edge of the tube. The undisturbed core of the peat sample, having a definite unit volume equal to approximately 3 cubic inches (5 cm.^{3}), is then dropped into a small "ointment" glass jar provided with a tightly fitting aluminum screw-cap. From a series of determinations on peat profiles of southern states it was found that the procedure lent itself to an accurate determination of the volume-weight of different layers of peat in a profile and was important in order to interpret properly various peat soil phenomena in connection with plant responses.

Samples of peat from the entire profile depth were obtained by the use of the American peat sampling instrument which is a modification of the device

[2] The writer wishes to express his appreciation to Dr. Steinmetz of the University of Maine for aid rendered in sounding the Veazie peat area.

first designed by the Wisconsin Geological Survey. The instruments made in the mechanical laboratory of this Bureau consist of a cylinder of duraluminum or of brass, approximately 10 to 14 inches (25 to 35 cm.) long and with an inside diameter of ¾ inches (2 cm.). The cylinder is provided with a plunger cone-shaped at the lower end, and with a spring-catch near the upper end. The spring permits the withdrawal of the plunger from its enclosing cylinder at any desired depth. When locked in that position the instrument may be filled with a solid core of the respective peat layer by a downward movement. The cylinder protects the peat sample completely from any contamination and does not destroy its structure when the instrument is lifted out. Beginning at the surface downward, samples of peat are taken consecutively with the greatest care from distances either at every 6 inches (15 cm.) or 1 foot (30 cm.) from each other. The samples are placed into sterilized containers, glass vials, or any other holder, suitably marked, by forcing the spring catch and the plunger back into the cylinder. A number of 2-foot (60-cm.) sections of ½ inch (1.7 cm.) duraluminum or of steel pipe can be readily connected by couplings. The lengths permit an easy estimation of the depth reached from which the peat sample is required. The instrument is kept in a canvas case, carried by a strap over the shoulder, and is very easy to handle. Tools such as the Swedish chamber drill of the Hiller model, boring sticks, and augurs have not given satisfactory results.

For the correct determination of the plant remains which have contributed to the formation of different layers of peat, an ecological herbarium with emphasis upon the plant community as the vegetation unit, and a collection of representative types of peat material are indispensable. The different types of peat found in this country even at great distances apart show a striking correspondence with those which occur in Europe, and they appear to have been derived from units of vegetation which on the whole are ecologically related. The necessary condition for the comparison of sharply demarked differences between types of peat with corresponding series of peat materials in Europe is, of course, the botanical and ecological method. The system of classification described in Departmental Bulletin 802 and the photographic illustrations published in Departmental Bulletin 1419 or in von Post's recent paper (4) permit with a fair degree of certainty the identification of the more important types and mixtures of peat materials found over practically the whole of the United States, Canada, and Europe. However, a compilation of an illustrated handbook of plant remains, including microscopic forms in peat deposits, is greatly to be desired and should be undertaken by the aid of international coöperation. Thus the main basis for coördination in peat investigations—reference of different kinds of peatlayers to their common origin and composition, and to one and the same standard of quality—would be rendered less difficult.

Preparations for microscopical examination of plant remains and their transition stages are handled in the laboratory in the following manner: Fresh ma-

terial is washed with distilled water and the smaller component parts of organic material are separated in water to which a few drops of chloral hydrate have been added. Air-dry peat samples are boiled in a 10 per cent solution of potassium hydroxide, essentially according to the method of Erdtman (3). Fractions of the peat material are prepared as microscopic slides in distilled water to which a drop of glycerine is added. Pollen grains and other minute plant remains are determined more or less quantitatively on samples which represent a fairly uniform layer of peat. The studies in pollen statistics require, however, much more development because the pollens of various species of deciduous plants resemble one another closely. Degrees of decomposition, following von Post's scale of ten divisions, are indicated as being poorly (H0-2), slightly (H2-4), partly (H4-6), largely (H6-8), and well (H8-10) decomposed. It is obvious, however, that a more exact quantitative method must be established to express the degree of decomposition that has taken place at different levels.

PEAT PROFILE RECORDS

Lewiston (Garcelon) heath

The topographical and geographical position of this deposit is shown on the Lewiston sheet of the U. S. Geological Survey. The area is located near the eastern city limits of Lewiston, Me. It exhibits a convex surface contour rising more than 5 feet (1.5 m.) from margin to center. The borders had at one time a considerable growth of spruce and tamarack with birch and alder but much of the timber has been cleared off by cutting and repeated fires. The conifers of the boreal climax are being replaced by white pine, red maple, and a dense growth of deciduous shrubs. Root sprouts of *Populus tremuloides* are occasional. The evergreen heath consocies in the central part of the peat area is dominated by *Chamaedaphne calyculata*, averaging between $1\frac{3}{4}$ to $2\frac{3}{4}$ feet (53 to 83 cm.) but *Kalmia glauca* and *K. angustifolia* are giving the locality a distinctive aspect in many places. Minor elements are *Ledum groenlandicum*, *Andromeda polifolia*, *Aronia nigra*, as well as *Spiroea* and *Solidago*. The chief element in the ground-cover consists of Polytrichum mosses (*P. strictum*, *P. commune*) favored by fire, and several lichens (*Cladonia rangiferina*, *C. uncialis*, *C. sylvatica*), while low small cushions of sphagnum mosses, chiefly mixtures of *S. tenellum*, *S. acutifolium*, *S. fuscum*, and others, occur in protected places but much suppressed.

The area has a marginal drainage ditch and a portion of it was formerly exploited in an attempt to manufacture peat fuel briquets. The drainage factor does not appear to have brought about much change in the ericaceous heath shrub complex but the deposit shows evidences of having shrunk vertically and compacted.

The profile sounding taken in the western half of the heath, about midway between the wooded border and the center is as follows:

1. 0–4 inches (0–10 cm.) very dark brown, largely decomposed, crumbly peat from Sphagnum and Polytrichum mosses with an admixture of ericaceous woody fragments and fibrous roots and culms of cotton-grass(*Eriophorum* sp.) moderately moist.

4–16 inches (10–40.5 cm.) light reddish brown, poorly decomposed fibrous, matted, moist Sphagnum peat (*S. cuspidatum*, *S. tenellum*, etc.) with an interlacing network of rhizomes and roots of the living surface vegetation.

16–20 inches (40.5–50.8 cm.) dark grayish brown, partly decomposed Sphagnum moss peat with fibrous culms and roots of cotten grass (*Eriophorum* sp.), small amounts of ericaceous woody fragments and finely divided organic debris; moist, firm, on

20–30 inches (50.8–76.2 cm.) dark reddish brown, wet Sphagnum moss peat, partly fibrous with moderate amounts of woody material from decaying branches and roots of shrubs and occasional conifers.

30–72 inches (76.2–182 cm.) reddish brown Sphagnum moss peat, in large part *S. cuspidatum* and *S. fuscum*, wet, compacted, more or less layered, contains at various levels small amounts of coarsely fibrous culms of cotton grass and coniferous root and stem fragments.

2. 72–86 inches (182–218.5 cm.) dark brown partly disintegrated ericaceous woody material, moist, compact, with admixture of partly fibrous moss and sedge peat. Marginal portions of this layer contain well-preserved leaves and slender woody fragments from Chamaedaphne and other heath shrubs, over

86–96 inches (218.5–243 cm.) dark brown to reddish brown, wet, partly decomposed fibrous moss peat (*S. fuscum*, with interlacing roots of woody perennials; *S. cuspidatum* etc.) marsh gas given off abundantly.

3. 96–120 inches (243–304 cm.) reddish to grayish brown coarsely fibrous, matted reed (Phragmites) peat on finely fibrous radicellate sedge-reed peat representing a former floating mat; weak odor of H_2S.

4. 120–156 inches (304–400 cm.) grayish brown sedimentary organic suspension, firm, (Dy and Detritus-Gyttja) with rootlets of sedges and reeds, grading into an olive brown, finely divided, more or less colloidal organic debris (Plankton Gyttja) which contains seeds of herbaceous plants and pollen from conifers.

5. 156+ inches (400+ cm.) gray to bluish gray, soft, sticky clay, compact at lower level, reported to be glacial Lake Champlain clay.

The profile examination indicates that the primary layers consist of limnetic sediments from an ancient shallow lake which proably represents the remnant of a former valley lake of considerable size. Sedge and reed marches continued the accumulation as a result of a further fall of the water level, and a forest began to spread over the telmatic types of peat after the lake had dried up. The formation of sphagnum peat began at a later, moister, postglacial period, but at present sphagnum mosses no longer continue their growth and dominance in the area. The surface material resembles a dark colored muck-like organic debris, somewhat mineralized partly from fires, and exhibits a marked physical change from the parent peat layer.

Veazie heath

The location, acreage, and topography of this peat deposit are shown on both the Orono sheet of the U. S. Geological Survey and on the map which accompanies the 1909 U. S. Soil Survey report of the Orono area. The deposit appears to be unconnected with the more extensive area of peat at Pushaw lake. In surface contour it is moderately convex, rising only a few feet from the wooded margin to the open growth in the center.

The vegetation presents the aspect of scattered small islands of dwarfed spruce, tamarack, and birch as boreal dominants with the usual ericaceous heaths grouped around them. These islands grow directly upon sphagnum moss peat in which the long and ramifying roots are embedded a foot or two (30–60 cm.) below the surface. Between the islands are low hummocky masses of Sphagnaceae on which trailing and erect but small-sized evergreen shrubs are about equally distributed. In the hollows between the hummocks there is an open growth of two sedges, *Scirpus caespitosus* and *Eriophorum vaginatum*, the latter being more frequent in the wetter spots with *Sarracenia purpurea*, *Arethusa bulbosa*, and *Cypripedium* sp. The characteristic plants upon the bright red cushions of *Sphagnum tenellum* are *Vaccinium oxycoccus* and *Drosera rotundifolia*.

The greatly dwarfed heath shrubs are dominated by *Chamaedaphne calyculata*. The subdominants are *Kalmia*, *Ledum*, *Aronia*, *Betula*, and occasional clumps of *Gaylussaccia dumosa*. They rise out of a ground cover of mosses, chiefly mixtures of *S. fuscum*, *S. medium*, *S. acutifolium*, and *S. girgensohnii* with invading *Polytrichum* sp. in a sub-copious position.

Nearly all around the bog and along the highway where drainage conditions are improved spruce and larch are dominant, with birch, alder, and heath shrubs especially abundant. The trees are of ordinary size but in the marginal transition they become smaller, scattered, and on the central portion of the bog rarely exceeding a few feet in height.

Approximately 250 feet (75 m.) west of the Stillwater-Bangor highway, the following profile was recorded on a moderate rise in the open low, shrub cover:

1. 0–48 inches (0–120 cm.) light yellow-brown, fibrous, spongy matted, poorly decomposed, wet Sphagnum moss peat, chiefly from species found at the surface, slightly altered, grading into a somewhat darker colored material below the 10-inch level, with an open network of tough, slender roots, fibrous rhizomes, and stems of woody perennials. Marsh gas escaping from lower level.

48–60 inches (120–152 cm.) brown, partly fibrous Sphagnum-Eriophorum peat with an admixture of ericaceous coarse woody fragments, loosely matted, waterlogged.

60–96 inches (152–243 cm.) water pocket with brown, soft, more or less suspended organic debris (Detritus-Gyttja); firmer at lower level.

2. 96–136 inches (243–345 cm.) dark brown, partly decayed, woody peat from logs, branches, and roots of conifers, birch, and shrubby undergrowth, embedded in finely fragmented woody debris, grading into

3. 136–140 inches (345–355 cm.) grayish brown detritus with rootlets of woody plants and sedges, granular when dry, over clayey sedimentary organic debris (Clay-Gyttja) somewhat elastic, on

4. 140+ inches (355+ cm.) gray, compact, fine sand with rootlets of herbaceous and woody plants at upper level, mottled with light gray spots below.

This profile suggests that an open, grassy forest invaded the area and continued for some time the accumulation of woody peat. The transformation of the forested peat area into a sphagnum covered moss bog occurred probably

at a time of the rise and expansion of the water surface of neighboring lakes. Thereafter sphagnum continued its dominance and formed a more or less floating layer of light brown, loose, coarse textured peat, with no evidence of marked physical change below the present surface.

Denbo heath

The conspicuously dome-shaped convex surface which rises 15 to 20 feet (4.5 to 6 m.) from margin to center, and the small ponds encountered on the highest top-parts, form the outstanding features of this area of peat. It is located about 18 miles (28 Km.) northwest of Cherryfield and covers an acreage of several square miles.

Although on the whole, the surface vegetation is the same as that of the other areas described there are yet minor differences. The present heath consocies is dominated by *Chamaedaphne calyculata*. The sub-dominants are *Kalmia glauca*, *Ledum palustre*, *Andromeda polifolia*, and several species of *Vaccinium*. Minor elements are *Eriophorum vaginatum*, *Scirpus caespitosus*, *Rhynchospora alba*, *Aronia nigra*, *Gaylusssaccia dumosa*, *Solidago sp.*, and greatly dwarfed conifers scattered over the bog. The ericaceous heaths, which in the transitional forested margin may grow to 5 feet (1.5 m.) in height, average only 5 to 7 inches (12 to 17 cm.) on the raised part of the bog. The ground-cover shows abundant mats of different species of Sphagnum mosses with *Vaccinium oxycoccus*, *Drosera rotundifolia*, and *Sarracenia purpurea*, but Cladonia and Polytrichum in particular are suppressing them, indicating drier conditions.

About the small ponds occur nearly pure carpets of *Sphagnum tenellum*, *S. magellanicum*, *S. acutifolium*, with Scirpus and Eriophorum dominating in narrow transition belts. A further stage in the succession are the irregular-shaped islands of low, dwarfed spruce and tamarack, about 2 feet (60 cm.) tall, with a fringe of ericaceous heaths, showing a tendency to occupy the hollows that are made by knoll-like hummocks of *Sphagnum fuscum* and other mosses.

Fire has been occasional but there appears to be no tendency toward the establishment of a fire sere or subsere.

The vegetation which constitutes the more or less encircling forest is characteristically dominated by spruce, tamarack, and birch with an ericaceous undergrowth. In the transitional zone and on the rising slopes the trees and shrubs generally become smaller. The great dwarfing and scantiness of conifers distinguish the raised from the flat portions of the peat area.

The test boring nearly midway between the wooded margin and a pond on the dome-shaped surface gave the following profile record:

1. 0–5 inches (0–12 cm.) thin superficial layer of grayish brown, partly decomposed leafy litter and granular debris embedded in Sphagnum mosses on light yellow brown fibrous moss peat (derived mostly from living species), poorly decomposed, spongy matted, moist, with interlacing slender rootlets.

5–12 inches (12–30 cm.) reddish brown, partly decomposed Sphagnum peat (*S. recurvum?*,

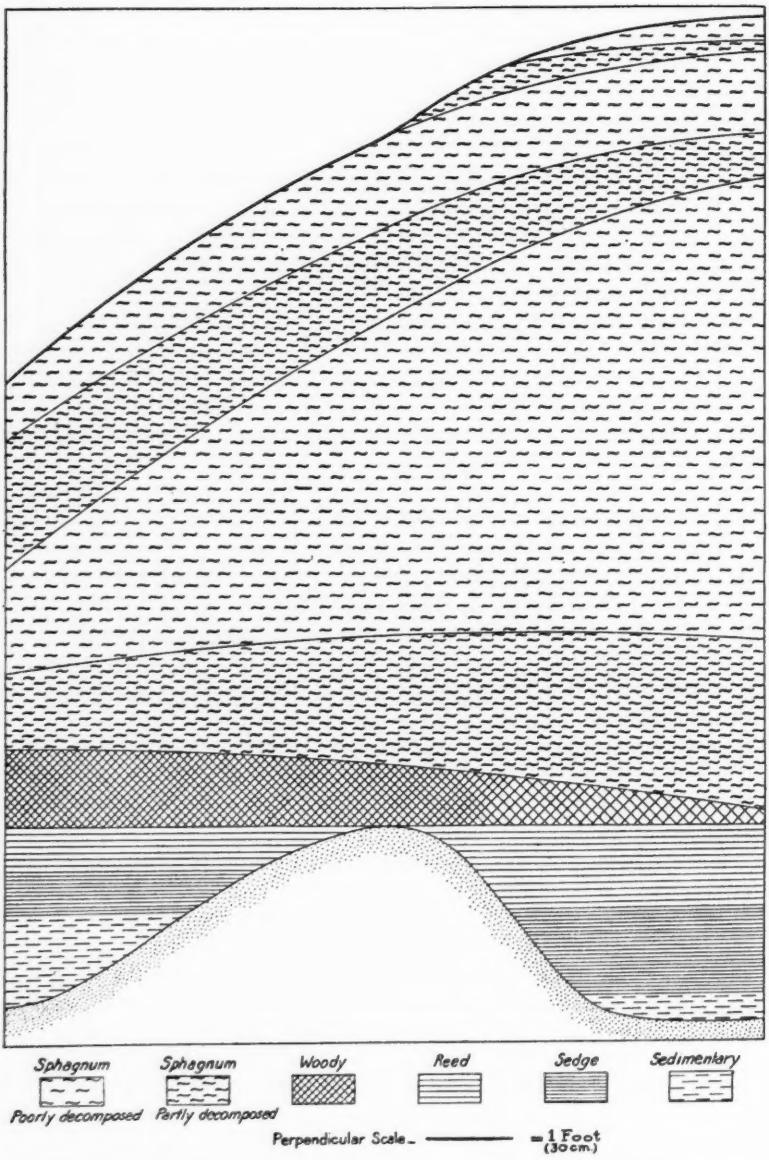

FIG. 1. CROSS SECTION OF A PORTION OF DENBO HEATH SHOWING MODE OF GROWTH AND DISTRIBUTION OF SUPERIMPOSED STRATA OF SPONGY FIBROUS MOSS PEAT WHICH HOLDS LARGE AMOUNTS OF WATER, ALTERNATING WITH MORE DECOMPOSED, COMPACT, AND DARKER COLORED STRATA OF SPHAGNUM MOSS PEAT

Compiled from series of profile soundings made between 1924 and 1928

S. imbricatum, and others), moist, somewhat firm, with embedded network of long tough woody stems and fibrous roots of *E. vaginatum* at lower level. Standing water fluctuating 10 inches (25 cm.) below surface.

12–28 inches (30–71 cm.) light reddish brown Sphagnum peat slightly decomposed, fibrous, loose, wet, with traces of course and fine rootlets of woody perennials, and occasional thin streaks of firmer, darker colored organic debris.

28–48 inches (71–121 cm.) brown, fairly compact Sphagnum peat, wet, in more advanced stage of decomposition than upper strata, with woody fragments of rather local distribution and abundance.

4–17 feet (121–518 cm.) light reddish brown to brown fibrous porous Sphagnum peat (includes mixtures of *S. fuscum*, *S. cuspidatum*, and others) poorly decomposed, wet, with open network of rootlets and occasional coniferous and ericaceous woody fragments, darker in color, firmer and partly decomposed below 13 foot (396 cm.) level; escape of marsh gas from various lower levels.

2. 17–18¾ feet (518–568 cm.) brown woody peat from branches, twigs, and poorly decomposed coarse woody roots of heaths and conifers, embedded as a tough interlacing network in waterlogged, finely divided organic debris; difficult to penetrate. Pollen of alder, birch, and spruce intermingled.

3. 18¾–22 feet (568–670 cm.) gray-brown, coarsely fibrous, matted reed (Phragmites) peat, containing ericaceous woody rootlets at upper level, forming a transition to radicellate sedge (Carex-Scirpus) peat, darker in color at lower level, firm, slightly moist, grading into

4. 22+ feet (670 + cm.) thin layer of brown to greenish brown sedimentary peat (Clay-Gyttja) with conifer pollen, on compact fine sand.

From a series of profile soundings which were made between 1924 and 1928 the cross section shown in figure 1 has been drawn to illustrate the various stages in the development of the dome-shaped convex form. It will be observed that darker colored, partly decomposed, compact, and relatively impervious moss substrata alternate with spongy, fibrous, poorly decomposed, light colored moss peat which holds considerable amounts of water in its capillary spaces. The whole layer of moss peat appears to have accumulated obviously under the influence of changes in atmospheric conditions and with distinct regularity: expanding masses which concentrated in the center, were followed by younger strata which diminished in extent with the growth in height. The transformation into a convex surface is on the whole, independent of any hollows, knolls, or ridges of the underlying sandy bottom. The features of the present surface vegetation point clearly to increasingly drier conditions, indicating that the peat no longer receives and absorbs a copious rainfall or condenses heavy fogs.

SUMMARY

The results presented in this paper bring out the following facts as to the essential characteristics of three peat profiles in Maine.

1. A consideration of the surface aspects shows light colored poorly to dark colored partly decomposed phases of Sphagnum moss peat varying in thickness from 5 to 17 feet (152 to 518 cm.), with a water table fluctuating between 3 to 10 inches (7.5 to 25 cm.) below the surface.

2. An examination of the internal composition and variation in profile structure indi-

cates that the layer of Sphagnum moss peat is superimposed upon a layer of woody peat moderately decomposed, followed in deeper depressions by a fibrous reed-sedge peat over sedimentary peat resting upon a clayey to sandy mineral substratum. Stratigraphically the peat profiles represent the conifer heath-sphagnum moss series and the sedimentary-reed sedge-conifer heath-sphagnum moss series.

3. The layers of peat are throughout under anaerobic conditions. The rate of decomposition is exceedingly slow at present, since the profiles show largely the inherent features of the component parent materials. In terms of stage of development (toward peat soil formation) the areas may be grouped into the category of immature, virgin peatlands, predominantly botanical in character.

4. Physiognomically the surface vegetation may be designated as shrubby heath-moor; it represents a successional stage passing into the conifer sub-climax.

5. The raised, dome-shaped surface is due chiefly to an upward mode of growth and periodic accumulation of Sphagnum mosses; it is probably related to a maritime climate (von Post's region of ombrogene peat deposits) which was until recently much moister than that now existing in the northeastern Atlantic coastal region. The partly decayed sublayers indicate probably the influence of temporary dry periods (2).

6. The areas of peat appear to have come into existence during a relatively recent period in post-glacial times. The general agreement in stratigraphic features probably relates to a common age of the deposits, the Lewiston peat being relatively older than the Veazie or the Denbo peat.

7. The peat areas may be included into a major division characterized on the one hand by the lack or the removal of nutrient mineral salts (low ash content) in any layer of peat or horizon near the surface (oligotrophic group of peat lands), and by the presence, on the other hand, of conditions which give to the organic material an acid reaction.

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CONTRIBUTION TO THE CHEMICAL COMPOSITION OF PEAT: IV. CHEMICAL STUDIES OF HIGHMOOR PEAT PROFILES FROM MAINE

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Peats are formed as a result of a certain one-sided decomposition of plant residues, in the presence of an excess of water, which makes conditions anaerobic and prevents the distinctly aerobic processes from taking place. However, if this were the only factor responsible for peat formation, the chemical composition of all types of peat would be alike. The fact that we have several types of peat which are distinct from one another in their chemical make-up points to other factors responsible for this difference. Among these, the nature of the vegetation and the environmental conditions under which decomposition is taking place, especially reaction and abundance of nutrients, are most important. Highmoor peats, lowmoor peats, forest peats, and sedimentary peats have certain characteristics in common, namely, the abundant accumulation of organic matter resistant to decomposition and a certain tendency toward a similarity in physical and chemical nature. But they are still distinctly different from one another, and can readily be recognized and well described, not only by their botanical composition as commonly assumed, but also by their chemical composition. A highmoor peat possesses certain definite characteristics, not merely because its plant associations are made up of various species of *Sphagnum*, *Eriophorum*, and various Ericaceous shrubs, but because these plants possess a definite chemical composition and because their chemical complexes will decompose only in a certain definite manner, especially at a high acidity and with a low mineral content, such as prevails in that type of bog. The lowmoor peat owes its specific composition not merely to the specific plants, but to the chemical composition of these plants and to the conditions under which they are undergoing decomposition. The same fact is true of the other types of peat.

In the decomposition of complex plant substances by microörganisms, the water-soluble substances are among the first to be attacked, and are followed soon by the pentosans and celluloses. The lignins are most resistant to decomposition, although it has been definitely established that at least some fungi belonging to the Hymenomycetes and possibly certain bacteria and actinomyces are capable of attacking lignins; the specific fungi may decompose these as rapidly as if not more so than they do the celluloses. However, this

process can take place only under aerobic conditions, whereas under anaerobic conditions, as in the case of the peat bogs, the lignins are hardly decomposed at all. Klason (9) many years ago was the first to express ideas of this nature when he stated that the processes whereby dead plants are changed into the first stages of peat must consist in the fermentation of carbohydrates; when this takes place in the absence of air, or when the organic matter is covered with water, the process must stop here, since the microörganisms active under these conditions are unable to ferment the other plant constituents; peat was thus considered to consist of lignin-like substances of plant origin in a more or less unchanged condition.

But the mere assumption that the formation of peat from plant residues consists in a gradual decomposition of the pentosans and other hemicelluloses as well as of the celluloses, and an accumulation of lignins or of lignin transformation products (so-called "humic acids") does not explain the marked differences in the chemical composition of the various peats as well as in the decomposition processes. Highmoor peat is characterized by a low ash content, by a high cellulose, fat, and wax content, by a fairly large amount of hemicellulose, and by a low protein content. The distinguishing characters of a lowmoor peat are the practical freedom from true celluloses, an abundance of proteins, a high mineral and lignin content, and a very low fat and wax content.

Analyses of various horizons of a highmoor peat profile from Germany were reported elsewhere (15). These results led to the conclusion that the formation of this type of peat is accompanied by a rapid decomposition of some of the nitrogenous complexes of the plant residues and a slow decomposition of the carbohydrates; these include not only the lignin-like complexes, but also the hemicelluloses and celluloses, as well as waxy substances. In the lowmoor peat formations, the reverse is true, namely, a gradual accumulation of the nitrogenous complexes and a rapid disappearance of the celluloses and hemicelluloses take place; the lignins accumulate but the ether-soluble substances do not.

Oden and Lindberg (12) reported that although Sphagnum peat contains 4.3 to 6.6 per cent ether-soluble substances and 3.3 to 4.7 per cent cellulose, Carex peat contains none at all or very little of either of these two groups of complexes. Hesz and Komarewsky (8) isolated 10 per cent cellulose from air-dry peat, presumably of the highmoor type; the same peat contained 6 per cent ether-soluble, 10 per cent alcohol-soluble, and 49 per cent material soluble in cold 1 per cent NaOH solution, including the pentosans, gums, pectins, etc.

Although Cajander (4) and others were largely justified in their assumption that chemical analyses of peat are worthless unless their botanical composition is well known, we possess also definite information that not only does the botanical composition of peat depend entirely upon the chemical conditions prevailing in the bog, especially the abundance of available nitrogen, calcium, and other minerals (16, 10), but that the two are closely interrelated.

Among the important factors which influence the vegetation in peat bogs, the absolute reaction occupies a prominent place. Olsen (13) found that when the pH of the bog is 4.2 or less, a true highmoor vegetation predominates, including *Eriophorum vaginatum, Oxycoccus, Calluna, and Andromeda.* With a decrease in pH value, the number of species diminishes. According to Brenner (3) typical sphagnum peat has a pH value of 3.9 to 4.5.

To throw further light upon the chemical composition and the nature of the organic complexes in the highmoor types of peat, three sphagnum bogs located in Maine were studied. A description of the location of these bogs, their botanical composition, as well as the profile features are published elsewhere in this issue of the journal by Dachnowski-Stokes.[1]

These three bogs were located as follows: 1. About eighteen miles from Cherryfield, Maine, and designated as *C*; this bog is known locally as the "Denbo heath" or "Deblois heath," near the town of Deblois; this is a typical highmoor bog rising in the center twenty to twenty-five feet from the outer margin; the bog has been described in detail by Bastin and Davis (1), Nichols (11), and Dachnowski (5).[2] 2. The second bog examined was located at the border of Pushaw Lake, a few miles southwest of Orono and also known locally as the "Veazie heath" or the "Orono bog" (designated as *O*). 3. The Garcelon bog (bog *L*) about two miles east of Lewiston, Maine, has been described by Bastin and Davis (1) and Dachnowski (5).

The following samples from these three bogs were used for chemical and bacteriological investigations:

Cherryfield bog:

C_1, Upper layer of the Cherryfield bog, at a depth of 2 to 8 cm., consisting largely of young *Sphagnum* peat with an admixture of some *Eriophorum* and various Ericaceous plants.

C_2, 8–20 cm. level, forming a darker layer, consisting also of *Sphagnum*, with an admixture of woody fragments from Ericaceous shrubs and *Eriophorum*.

C_3, 20–30 cm. level, reddish brown peat consisting of practically pure *Sphagnum*.

C_4, 30–46 cm. level, pure *Sphagnum* peat.

C_5, 46–61 cm. level, pure *Sphagnum* peat.

C_8, 183–214 cm. level, pure *Sphagnum* peat.

C_9, 460–480 cm. level, pure *Sphagnum* peat.

C_{10}, 550–580 cm. level, sedge peat, lowmoor formation.

Orono bog, about 330 cm. depth; no sedimentary layer at bottom; the bog resting upon forest formation. Surface vegetation consisting of *Sphagnum* (*Sph. squamosum, Sph. tenellum*), with an admixture of *Eriophorum, Saracena*, and various shrubs (*Ledum, Cassandra, Andromeda, Kalmia, Vaccinium*) as well as of tamaracks and spruces.

O_1, surface layer of peat, 1–10 cm. deep.

O_2, 15–20 cm. layer, light *Sphagnum* peat.

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[1] The sampling and general examination of these three bogs were carried out together with Dr. Dachnowski-Stokes of the Bureau of Chemistry and Soils, U. S. Department of Agriculture, to whom the authors are indebted for the helpful assistance rendered. The authors take this opportunity to express their indebtedness also to Dr. Steinmetz of the Maine Agricultural Experiment Station, who assisted in the location and sampling of the Orono bog.

[2] See also (7). Compare some of the analyses reported by Dachnowski (6).

O_3, 20–30 cm. layer, light *Sphagnum* peat.
O_4, 90–120 cm. layer, light *Sphagnum* peat.
O_5, 150–180 cm., *Eriophorum* and woody peat admixture.
O_6, 240–270 cm. layer.
O_7, 270–330 cm. layer, forest peat, bottom of deposit.

Lewiston bog found to be about 425 cm. deep, at the place of sampling, with a marked sedimentary layer at the bottom of the profile.

L_1, Surface 1–10 cm. level, consisting of *Polytrichum-Eriophorum-Sphagnum* peat.
L_2, 20–25 cm. level, *Sphagnum* peat.
L_3, 40–50 cm. level, *Sphagnum* peat.
L_4, 60–75 cm. level, *Sphagnum* peat.
L_5, 240–270 cm. level, sedge peat.
L_6, 360–400 cm. level, sedimentary peat, with a clay underground.

The moisture content of the original peat samples and the reaction, as expressed in terms of hydrogen-ion concentrations (determinations made by means of a quinhydrone electrode using Leeds and Northrup Type K potentiometer) are given in table 1. The samples were immediately air-dried as soon as brought to the laboratory and chemical analyses were made on the air-dry material. The ash and nitrogen content of the different samples are also given in table 1.

The results show that the typical sphagum peats are, in agreement with the results obtained by other investigators, highly acid in reaction, having a pH of 4.0 or even less. The *Sphagnum* layers are low in ash and in nitrogen. When the sedge peat is reached, there is an immediate rise in the pH value and in the ash and nitrogen content. In other words, the higher the acidity of the bog the less is its nitrogen and ash content. It is important to note that the minerals are usually more concentrated at the surface of the bog than in the lower levels, until the lowmoor or forest horizons are reached. Birk (2) also found previously that, although the ash content of the peat in the profile diminishes from the bottom toward the surface, there may be another rise near the very surface.

However, the three bogs used in these investigations show considerable differences in this connection, depending entirely upon their topography and vegetation.

The Cherryfield profile, which is the most typical highmoor peat, shows in the upper layers a variation in pH value from 3.73 to 4.05; there is a gradual decrease in acidity at a depth of 180 cm. reaching in the horizon of the sedge peat a pH value of 5.18. The ash content varies in the upper sphagnum layers from 0.90 to 1.14 and is only 2.00 per cent at the immediate surface, but there is a considerable increase in the sedge layer to 2.83. The nitrogen varies in the sphagnum layers from 0.653 to 0.839 per cent, but in the sedge layer the nitrogen has increased to 1.845 per cent.

In the Orono peat, the reaction is somewhat less acid, varying in the sphagnum peat horizons from pH 3.95 to 4.35, increasing rapidly to pH 5.70 in the sixth layer and to 6.04 in the lowest forest layer. The somewhat higher pH

value is accompanied by a higher ash content, ranging from 1.45 to 3.05 per cent in the sphagnum peat horizons, with an increase to 8.01 per cent in the sedge peat horizon and to 9.34 per cent in the lowest layer of the forest peat. The nitrogen content is just as low in the sphagnum horizons of this bog as in those of the previous bog (0.635 to 0.915), but there is a rapid increase in nitrogen in the woody-*Eriophorum* layer, even greater than in the sedge peat layer.

TABLE 1

Moisture content, reaction (pH), ash, and nitrogen content of various layers of three highmoor peat profiles

SAMPLE NUMBER	DEPTH OF LAYER	MOISTURE CONTENT OF ORIGINAL SAMPLE	REACTION OF FRESH PEAT	ASH CONTENT OF DRY MATERIAL	NITROGEN CONTENT OF DRY MATERIAL
	cm.	*per cent*	*pH*	*per cent*	*per cent*
C_1	1–8	92.7	4.05	2.00	0.653
C_2	8–20	92.6	3.95	1.14	0.684
C_3	20–30	92.6	3.85	1.02	0.817
C_4	30–46	92.9	3.86	0.90	0.839
C_5	46–61	93.6	3.73	1.06	0.770
C_8	183–214	93.4	4.47	0.99	0.705
C_9	460–480	92.4	4.71	1.10	0.756
C_{10}	550–580	92.2	5.18	2.83	1.845
O_1	1–10	94.2	4.35	3.05	0.635
O_2	15–20	93.9	4.30	2.80	0.646
O_3	20–30	91.8	3.95	1.84	0.915
O_4	90–120	93.7	4.13	1.45	0.885
O_5	150–180	95.0	4.20	1.74	2.105
O_6	240–270	92.6	5.70	8.01	2.151
O_7	270–330	89.9	6.04	9.34	1.931
L_1	1–10	88.3	4.10	8.79	1.135
L_2	20–25	93.6	3.99	1.85	0.683
L_3	40–50	92.8	3.98	1.68	0.924
L_4	60–75	90.0	4.15	1.25	1.240
L_5	210–240	90.1	6.28	4.24	.....
L_6	360–400	92.1	6.86	23.58	2.911

The Lewiston peat shows again somewhat different relationships due to the difference in the nature of the plant associations that have contributed to the make-up of this bog: horizons 2 to 4, which are typical sphagnum layers, have a pH value of 3.98 to 4.15, an ash content of 1.25 to 1.85, and a nitrogen content of 0.640 to 1.240; the very surface layer of the peat is rich in ash (8.79 per cent) and in nitrogen (1.135 per cent), but it is also fairly acid (pH 4.10). There is a rapid decrease in acidity in the sedge horizon and especially in the sedimentary peat, where it reaches nearly the neutral point; a parallel increase

is observed in the ash content. The nitrogen content of the peat also increases with depth, especially in the sedimentary peat.

The chemical analyses of the various horizons of these three peat profiles have been carried out according to the methods outlined previously (14). The results are reported in tables 2 to 4.

The chemical complexes found in the eight different horizons of the Cherryfield bog (table 2), taken at various depths from the surface to a depth of 20 feet, show all the characteristics of a highmoor peat. This is brought out by the large amount of fats and waxes (ether-soluble fraction), by the high cellulose and hemicellulose content, and by the low protein and ash content. The ether-soluble and alcohol-soluble substances increase with depth, the hemicelluloses and celluloses decrease, and the lignin-like complexes increase. It is interesting to note that the change from the sphagnum peat to the sedge peat in the deepest sample taken (C_{10}), which is characterized by an

TABLE 2

Chemical composition of a highmoor peat profile located near Cherryfield, Maine

On per cent basis of dry material

HORIZON NUMBER	ETHER SOLUBLE	ALCOHOL SOLUBLE	HEMI-CELLULOSES	CELLULOSES	LIGNINS	PROTEINS	ASH	TOTAL
C_1	2.35	1.45	26.45	16.86	27.18	4.08	2.00	80.37
C_2	2.62	1.92	25.24	14.74	29.21	4.28	1.14	78.95
C_3	2.82	1.83	24.55	15.97	28.85	5.11	1.02	80.15
C_4	2.57	2.08	22.25	13.69	32.23	5.24	0.90	78.96
C_5	2.96	3.20	18.48	14.66	33.24	4.81	1.06	78.41
C_8	3.97	3.15	15.94	15.55	37.43	4.41	0.99	81.44
C_9	4.89	4.29	12.69	11.85	44.83	4.73	1.10	84.38
C_{10}	5.97	5.06	5.96	5.06	54.11	11.53	2.83	90.52

increase in pH value, in the nitrogen and in the ash content, shows also a considerable drop in the abundance of celluloses and hemicelluloses and a rapid increase in the lignin content. These phenomena are characteristic of lowmoor peat formations in typical lowmoor profiles. When the chemical composition of this highmoor profile from the extreme northeastern portion of the United States is compared with the composition of the highmoor peat profile obtained in Germany, as recorded elsewhere (15), it is found that the two are very much alike.

In other words, independent of the region, topography, and history of the bog, the chemical composition of a definite layer in a peat profile depends entirely upon the nature and chemical composition of the vegetation from which this layer originated and the conditions under which its decomposition has been carried out.

Just as a thin layer of sphagnum peat found in a forest peat profile (15) had all the chemical characteristics of various layers of sphagnum peat in typical

highmoor profiles, so does a layer of sedge, of forest, or of sedimentary peat in a highmoor peat profile possess all the chemical characteristics of similar layers in lowmoor peat profiles or sedimentary peats. In other words, these results show conclusively that peats, considered not only from the point of view of the profile as a whole but also as a series of different layers varying in origin and composition, can be characterized by their chemical composition; this may have even a more important bearing upon the applications to be made of these peat formations, than a knowledge of their botanical composition alone. To be able to characterize a peat both by its botanical and chem-

TABLE 3

Chemical composition of a sphagnum peat profile near Orono, Maine

On per cent basis of dry material

HORIZON NUMBER	ETHER SOLUBLE	ALCOHOL SOLUBLE	HEMI-CELLULOSES	CELLULOSES	LIGNINS	PROTEINS	ASH	TOTAL
O_1	1.76	1.40	26 30	16.43	19.15	3.97	3.05	72.06
O_2	2.53	1.39	25.51	13.33	22.23	4.04	2.80	71.83
O_3	2.45	1.90	20.92	16.23	25.43	5.72	1.84	74.49
O_4	2.57	2.20	22.68	12.07	25.83	5.53	1.45	72 33
O_5	3.63	2.83	15.78	10.84	35.75	13.15	1.74	85.01
O_6	2.60	2.16	5.93	3.20	52.79	13.44	8.01	88.13
O_7	2.73	2.06	4.78	2.70	54.94	12.07	9.34	88.62

TABLE 4

Chemical composition of a sphagnum peat profile at Lewiston, Maine

On per cent basis of dry material

HORIZON NUMBER	ETHER SOLUBLE	ALCOHOL SOLUBLE	HEMI-CELLULOSES	CELLULOSES	LIGNINS	PROTEINS	ASH	TOTAL
L_1	2.90	2.10	18.09	9.72	26.96	7.09	8.79	75.65
L_2	1.73	1.37	26.57	14.06	21.49	4.27	1.85	71.34
L_3	4.83	2.75	13.82	11.40	35.85	5.78	1.68	76.11
L_4	3.55	3.81	11.15	9.08	47.47	7.75	1.25	84.06
L_5	2.24	1.86	5.60	1.40	37.44	18.19	23.58	90.31

ical composition is of course of even greater significance for gaining an understanding of bog formations and their practical utilization.

Table 3 gives the analyses of the Orono peat profile. In this bog as well, the upper four horizons consist largely of sphagnum peat which is characterized by a high acidity, a low ash and nitrogen content, a high hemicellulose and cellulose content, and a rather low lignin content. The fifth horizon (O_5) forms a transition to the sedge layers of peat. This is characterized by a slow but gradual increase in pH value, a rapid increase in the nitrogen, a gradual but marked decrease in the celluloses and hemicelluloses, and a definite increase in the lignin-like complexes.

The lowest two horizons of this profile consist of sedge and forest peat. In chemical composition, these two layers are found to be typical of sedge and forest peats. This is brought out by a rapid decrease in acidity or increase in pH value, a decided increase in the ash content, a high nitrogen content, a decrease in the celluloses and hemicelluloses, and an increase in the lignins. A definite parallelism is thus found again between the acidity of the peat formation and the cellulose and hemicellulose content, while an inverse relation is found to exist between these and the high ash, protein, and lignin content. A high acidity (low pH) indicates a characteristic growth of *Sphagnum* and *Eriophorum*; it indicates the presence of certain types of celluloses and hemicelluloses and their slow decomposition; it indicates a low protein content due to insufficient activities of the microörganisms. On the other hand, a low acidity points to a vegetation of grasses, to a rapid decomposition of the hemicelluloses and the celluloses, and to an accumulation of the lignins, proteins, and minerals.

An examination of the results obtained in the analysis of the various horizons of the Lewiston peat profile tends to confirm fully the above considerations. Here as well, the upper four horizons are markedly acid in reaction and represent a peat formed by a mixed vegetation of *Sphagnum* and Ericaceous shrubs. In chemical composition, these horizons are very similar to the upper corresponding horizons of the Orono profile, with certain distinctive differences due of course to the fact that both the vegetation and the conditions of decomposition are somewhat different in the two bogs. The sphagnum horizons are highly acid, have a low ash (except the surface layer) and nitrogen content, a high cellulose and hemicellulose content, and a low lignin content; the lowest or the fourth of these horizons (L_4), still acid in reaction and low in ash, shows already a marked diminution in the hemicellulose and cellulose content and an increase in lignins. When the sedge and sedimentary horizons are reached, there is a decrease in acidity, an increase in ash and nitrogen content, a decrease in celluloses and hemicelluloses, and an increase in the lignins.

This third profile is not a typical highmoor as the Cherryfield profile is and as the German peat profile described previously was. It represents a certain intermediary stage in the nature of its vegetation; it does not show as distinct differences in the chemical composition as do the typical highmoor and lowmoor profiles. However, the chemical composition of the various horizons in even such an uncharacteristic profile throws definite light upon the nature of peat formations.

Attention is here again called to the fact that in the lowmoor and forest peats, as well as in the sedge, forest, and sedimentary layers of highmoor peats, the sum total of the various organic and inorganic complexes accounted for in the proximate chemical analysis is about 90 per cent and frequently more. However, in the sphagnum peats the sum total of the chemical constituents accounted for by this method is only 72 to 80 per cent. This is largely because

in the treatment of the peat with 2 per cent hot hydrochloric acid, following the treatment with ether, alcohol, and water, about 50 to 55 per cent of the organic constituents are brought into solution; but of this, only about a half is accounted for in the form of reducing sugars and recorded as hemicelluloses (after multiplying the reducing sugar by 0.9). Since the nitrogenous complexes and the ash fraction brought into solution by the dilute acid do not in account for more than 2 to 4 per cent of this extract, 20 to 25 per cent of the organic constituents of the sphagnum peat which are soluble in hot dilute acids, but which do not give any reducing sugars, remain unaccounted for. This fraction includes various pectins and gum-like complexes, which give on hydrolysis organic acids and other non-reducing substances. It may also include certain polysaccharides which give furfural even on boiling with dilute acid and which are lost therefore from the determination. A study of the chemical nature of this complex will soon be undertaken.

A large number of anaerobic bacteria were found in the various horizons of the profile. These bacteria are largely anaerobic in nature although many of them are facultative anaerobes and can, therefore, live also under aerobic conditions. These organisms were found in an active state and their numbers do not diminish with the depth of the bog but even tend to increase. They bring about the decomposition processes taking place slowly in the bogs. A detailed study of the nature of these organisms and their activities will be published in a subsequent contribution.

SUMMARY

A study has been made of the chemical composition of the organic constituents of three sphagnum peat bogs in Maine, one of which was a typical highmoor bog.

The sphagnum horizons are very acid in reaction, of a pH around 4.0. The high acidity is always accompanied by a low ash (except sometimes at the very surface of the bog) and nitrogen content; a high cellulose, hemicellulose, fat, and wax content; and a low lignin content.

With the transition of the sphagnum layers into a sedge, forest, or sedimentary peat layer, there is an immediate rise in the pH value, an increase in the ash, a decrease in the cellulose and hemicellulose, and an increase in the protein and the lignin contents.

The chemical composition of the organic and inorganic complexes of peat bogs is quite sufficient for the description of these bogs, and a knowledge of this composition is of special importance from the point of view of the practical utilization of the bogs. When this information can be combined with a careful botanical description of the vegetation which has contributed to the formation of the various horizons, the results are of even greater significance for our understanding of the nature of the peat formations, their origin, and the methods of handling when brought under cultivation.

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THE EFFECT OF SWEET CLOVER AND ALFALFA ROOTS AND TOPS ON THE FUNGOUS FLORA OF THE SOIL

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Investigations on the influence of specific kinds of materials on the development of soil fungi have been rather limited. Some phases of the subject of soil mycology and of the part that soil fungi play in the economy of the soil are only in the beginning stage. Enough work has been done, however, to indicate that soils changed by the addition of various materials which influence bacterial content and activity, also influence changes in soil fungi (5). Waksman (6) quotes the results of some investigators in which it was found that the addition of stable manure stimulated the development of *Penicillia* and *Mucorales*, and that pure cellulose in the presence of available nitrogen brings about an extensive development of *Trichoderma*, *Fusarium*, *Verticillium*, *Monosporium*, and certain *Penicillia*. The writer (3) found *Mucor*, *Rhizopus*, and *Alternaria* predominating in alfalfa root treated soils, *Cladosporium* in straw treated soils, and *Aspergillus* and *Penicillium* in sweet clover root treated soils.

The previous work seems to indicate that different kinds of organic materials influence to some extent the types of soil fungi which are most active in the soil. In previous studies by the author, sweet clover and alfalfa tops and roots seemed to differ in substance enough to warrant further study, so the experiment described below was planned and carried through.

PLAN OF THE EXPERIMENT

One-gallon glazed jars were filled with a clay loam soil of pH 7.5. The soil used was secured at a depth of 6 feet in the spring of 1928, and was left exposed until September, 1928. No plant material had obtained a foothold. Very slight summer showers were all the moisture whieh the soil had received. The surface 2 inches of a pile of this soil was removed and the soil used for the experiment was secured from this depth down to 12 to 18 inches. One per cent of air-dried ground alfalfa roots having a total nitrogen content of 1.33 per cent was added to each of three jars. Similar jars of soil were treated with corresponding amounts of dried and ground alfalfa tops, sweet clover tops, and sweet clover roots having a nitrogen content of 2.25 per cent, 2.30 per cent, and 0.77 per cent respectively. Three jars of soil were used as controls. The alfalfa roots were obtained to a depth of 12 inches from a 5-year-old alfalfa field and the tops were taken from the first crop at about the half mature stage.

The sweet clover roots were obtained to a depth of 12 inches from a second-year growth of sweet clover and the tops were approximately one-third grown. The moisture content of the soils was then brought to 25 per cent dry basis. All of the jars were kept under laboratory conditions at room temperatures varying from 19° to 21°C.

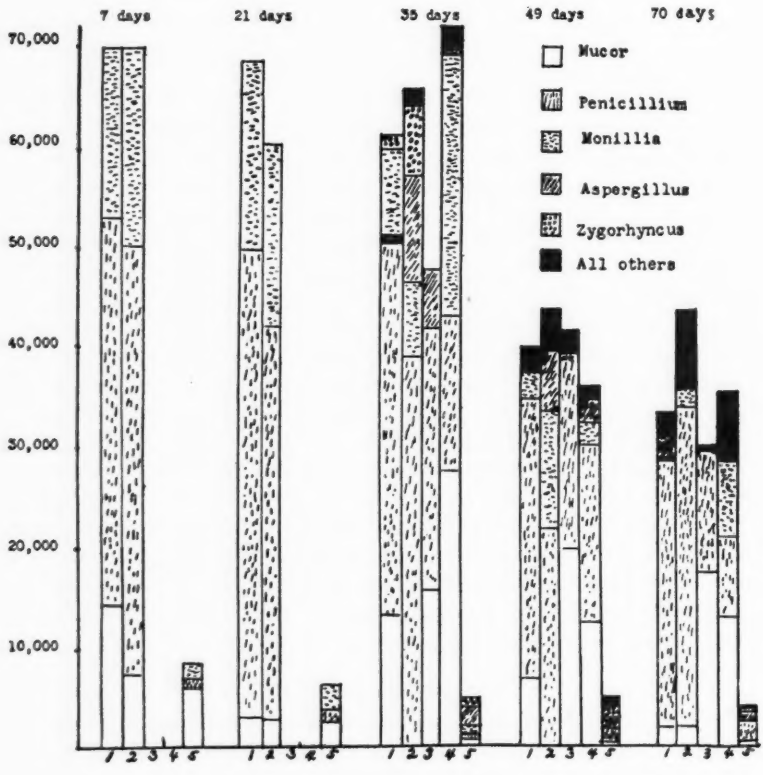

FIG. 1. GRAPH INDICATING THE RELATIVE DEVELOPMENT OF FUNGI AT DIFFERENT PERIODS OF TIME

Columns 1, 2, 3, 4, and 5 represent sweet clover roots, alfalfa roots, sweet clover tops, alfalfa tops and checks, respectively.

At intervals of 1, 3, 5, 7, and 10 weeks, the soils were sampled and mold counts and identification of mold genera were made for each sample. As previously mentioned, each soil was treated in triplicate. Four plates were poured from each sample, thus making 12 plates for each treatment. The average of each 12-plate group is given in the tables. Raisin agar described by Waksman (4) was used as the medium. The identification of the molds

produced on the plates was made according to the descriptions given by Buchanan (1), Gilman and Abbot (2), and Waksman (7).

DISCUSSION

It will be noticed that no counts are given in table 1 for the 7- and 21-day periods. When the plates were examined at the end of 3 days, they were so completely overgrown with species of *Mucor* that it was impossible to make counts. At the end of the 35-day period, the *Mucors* grew more slowly and the mycelium filled the plates less densely. It was, therefore, possible to make counts and study the types developed. The Penicillium colonies were very small. The results obtained at the 49-day period indicated a further retardation in the development of *Mucors*. There was, however, a slight increase in numbers. There was also a greater variation in the types developed. The 70-day period showed a very slow growth of *Mucors*. The Penicillium colonies were larger but fewer in number. The total number of molds for each period of determination diminished as the time of incubation increased.

As indicated in table 2, the sweet clover roots had a different influence on mold development. During the first period, the *Mucors* developed in larger numbers but their growth was very slow compared to the sweet clover top treatments. There were many large colonies of Monillia at first, but after 35 days, they decreased rapidly. The *Penicillium* colonies were the predominant fungi for clover roots in every period. Their numbers tended to decrease with time, but not so rapidly as did the Monillia and the *Mucors*. For some reason, the *Mucors* did not show much development during the 21-day period, particularly when compared with the 35-day period. The Monillia and Penicillia were about the same as in the previous sampling. In 35 days, the variation in types was more marked but all the colonies were very small. In the 49-day determination, it was found that the numbers had diminished, the *Mucors* grew more slowly and the Monillia were relatively quite large. The 70-day period failed to show much change in rate of growth and size of colonies. There were fewer fungi but the decrease occurred in all types, although the Penicillium showed the least relative change. There was also a slightly greater variation in mold type. As the incubation period lengthened the total number of colonies decreased in numbers, the same was noted for the tops, but the tops maintained its *Mucor* growth while the roots declined. Another significant difference is that the clover tops supported almost no Monillia, but the roots showed at least some in every period. The flora of the clover tops is largely confined to the *Mucor* and Penicillium types and is not nearly so variable as the tops.

Table 3 shows that alfalfa tops had about the same influence as sweet clover tops on *Mucor* development for the first 21 days of incubation. It was impossible to make counts because of the vigorous and rapid growth of the *Mucors*. The 35-day period indicates a predominance of *Mucors*, but they grew more slowly and did not fill the plates with mycelium. Monillia were

TABLE 1

Effect of sweet clover tops incorporated with the soil on the development of numbers and types of soil fungi

Number per gram of soil and average of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	.....	.....	48,000	43,000	32,812
Zygorhynchus	Plates overgrown with *Mucor* in 3 days		.....	280	.....
Monillia			.....	1,200	312
Aspergillus			6,000	270	.....
Penicillium			23,000	20,000	12,500
Mucor			19,000	21,250	20,000

TABLE 2

Effect of sweet clover roots incorporated with the soil on the development of numbers and types of soil fungi

Number per gram of soil and average of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	70,000	68,000	64,400	43,150	48,300
Sporotrichum	.....	.....	.....	.....	2,500
Botrytis	.....	.....	200	3,250	.....
Zygorhynchus	.....	.....	1,250	.....	1,000
Monillia	17,500	18,000	7,500	3,625	200
Aspergillus	.....	.....	400	.....	1,500
Penicillium	37,500	47,500	37,500	29,000	26,250
Mucor	15,000	2,500	14,500	7,250	2,750

TABLE 3

Effect of alfalfa tops incorporated with the soil on the development of numbers and types of soil fungi

Number per gram of soil and average of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	.....	.....	72,450	36,780	38,650
Botrytis	Plates overgrown with *Mucor* in 3 days		.....	.....	200
Alternaria			.....	.....	8,200
Trichoderma			.....	1,250	.....
Rhizopus			4,000	.....	.....
Aspergillus			200	2,500	500
Monillia			25,000	2,500	6,500
Penicillium			16,250	16,250	7,500
Mucor			27,000	14,280	15,750

very noticeable in their development. All the other colony types were small; particularly was this the case with the Penicillium colonies which ranked third in number of colonies. At the end of the 49-day period, Rhizopus developed as also did the Trichoderma, although in total numbers, they did not approach the kinds mentioned above. The *Mucors* grew more slowly. At the end of 70 days, the *Mucors* continued to diminish in vigor, although not in numbers.

TABLE 4

Effect of alfalfa roots incorporated with the soil on the development of numbers and types of soil fungi

Number per gram of soil and average of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	70,000	61,000	60,500	47,000	17,800
Botrytis	.....	.....	.....	3,500	.....
Trichoderma	.....	.....	.....	1,250	550
Alternaria	.....	.....	300	300	.....
Zygorhynchus	.....	.....	6,500	7,500	.....
Rhizopus	.....	.....	1,115	300	8,750
Aspergillus	.....	.....	11,750	.....	.....
Monillia	20,000	18,000	7,750	11,750	2,500
Penicillium	42,500	40,500	39,200	22,500	32,500
Mucor	7,500	2,500	.....	.....	2,500

TABLE 5

Numbers and types of soil fungi developed in soil without treatment with organic matter and used as controls for this experiment

Number per gram of soil and averages of 12 plates reported

	7 DAYS	21 DAYS	35 DAYS	49 DAYS	70 DAYS
Total	7,900	7,550	4,055	4,740	3,200
Trichoderma	.....	.....	.....	600	.....
Zygorhynchus	.....	.....	1,125	1,000	200
Aspergillus	.....	.....	2,000	1,040	800
Rhizopus	.....	.....	.....	300	.....
Monillia	1,250	3,700	330	800	.....
Penicillium	400	1,250	400	800	2,000
Mucor	6,250	2,600	200	200	200

The other types grew fairly large. There was a decrease in numbers as the length of time of incubation increased. This decrease was due to a diminution in all three of the main types of fungi but especially in the Monillia.

Table 4 presents the results following alfalfa root incorporations. The *Mucors* during the first period were few in number and their growth was very slow. The Penicillia grew quite rapidly and the colonies were relatively large

compared to the previous organic matter treatments. The Monillia grew more vigorously than in any of the previous treatments, with the possible exception of alfalfa tops. The 21-day samples indicated a very similar growth to the first week, the *Mucors* being fewer and slower in growth. The 35-day determinations showed a greater variation in colony types. There was no *Mucor* growth. The Monillia colonies were the largest that had been noticed thus far in the investigation. The 49-day period, as in the previous one, failed to show any *Mucor* development. There was a greater variation in types and the Monillia were the predominating colonies on the plates. The 70-day studies indicated a growth very similar to the 49-day determinations. The numbers had decreased more than previously.

The results from the controls, given in table 5, show a very poor growth of molds. The colonies were very slow in growing and the numbers were very few compared to the treated soils. During the 7-day period, the *Mucors* were small and the Monillia large. In three weeks the colony development had diminished. In five weeks there was a greater variation in types but fewer numbers. This was indicated also in the 7-week and 10-week determinations.

The most noticeable fact in all of these studies is that sweet clover tops and alfalfa tops stimulated a very vigorous *Mucor* development during the early part of the incubation period. The roots encouraged a greater variation in types. The Monillia colonies developed very noticeably in the alfalfa-treated soils, particularly with the root incorporations.

Comparing the number of colonies found in the two sources of organic matter, it is significant to note that the *Mucors* do not grow so abundantly in alfalfa as in clover. The only exception to this is for the tops during the 35-day period and the roots during the second period, when they were equal. The Monillia, on the other hand, are distinctly more numerous in the alfalfa treated soils, especially for the tops, and for the roots during the latter part of the experiment. The Penicillia do not show any well-defined reaction to either source of plant material, but are irregular at the different times when counts were made. On the whole, alfalfa tends to support a good growth of a wider variety of fungi than does clover, but during the first two periods when the greatest numbers of organisms were at work, the roots of both supported no recognizable fungi except *Mucor*, Penicillia, and Monillia.

Organic matter is a factor in mold development. One year ago, the writer attempted a similar study using a soil that had been cultivated for several years and had a nitrate content at the beginning of 40 p.p.m. The variation in types of organic materials added failed to show differences in mold development. The soil used in this experiment, however, never had grown a crop and the nitrate content at the beginning was 13 p.p.m. To study the influence of different kinds of organic matter on mold development, it is evident that a soil low in organic matter is essential. It may be that the nitrogen content of the organic material added is a distinct factor in the type of mold

development. The rate at which this nitrogen is made available also must be considered because in the soils when a vigorous nitrate accumulation occurred there was the greatest *Mucor* development.

CONCLUSION

When a clay loam subsoil is treated with alfalfa roots and tops and sweet clover roots and tops, these treatments influence the types of molds developed on that soil. The more succulent the material, the greater the stimulation to the development of *Mucors*. As the period of decay lengthens, there is a noticeable decrease in total numbers and also in the rapidity of development, particularly of the *Mucors*. The alfalfa root and sweet clover root incorporations do not influence the development as much as do the corresponding tops, but the roots increase the number of Penicillia more than do the tops.

Mucors grow more rapidly in soil containing either the tops or the roots of sweet clover than they do with incorporations of the corresponding parts of alfalfa, but the opposite may be said of the Monillia. The incorporation of alfalfa in a soil favors a wider variety of molds than does the incorporation of sweet clover. In general, the total number of colonies is greater in alfalfa treated soil than in sweet clover treated soil. After a period of 35 days, or after the subsidence of the vigorous growth of *Mucors* following the incorporation of the tops, the total number of colonies of molds is greater in soils treated with roots than it is in soils treated with tops of either alfalfa or sweet clover, and this is largely due to the greater number of Penicillia, although the roots also tend to have a greater variety of molds.

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THE GRAVIMETRIC METHOD FOR THE DETERMINATION OF CARBONATES IN SOIL

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Of the three methods generally used for the determination of carbonates in the soil—titrimetric, volumetric, and gravimetric—the gravimetric has fallen to a certain extent into disfavor and has received little attention for some time. Cain (5) cited many of the difficulties encountered in the absorption of carbon dioxide and the weighing of the absorbent, and soil chemists have not made any serious effort to improve the process, although it is generally accepted that the method gives very accurate results if precautions are observed (16).

The procedure can be stated briefly as the production of carbon dioxide from the carbonates by the action of dilute acid on the soil; the purification, drying, and absorption of the resulting gas, by drawing it through a "train" by aspiration; and the final weighing of the absorbing material. The chief precautions needed are: 1. the preliminary sweeping out of all traces of carbon dioxide from the apparatus; 2. the decomposition of all the carbonates in the soil without an attacking of the organic matter—a difficulty shared with the gas-volumetric and titrimetric methods; 3. the removal of the air from the absorption bulb with the same water content as it has on entering. Errors may also be incurred by the variation in the amount of moisture on the surface of the absorbing bulb. In the apparatus described hereafter, we have made an adaptation which shortens the time needed for sweeping out the carbon dioxide; the decomposition of the carbonates has been accelerated; the time taken both for the absorption and for the weighing has been materially reduced.

The old method of heating the soil with strong acid in order to set free the carbon dioxide was abandoned because of the action on the organic matter. Marr (10) at Rothamsted, after trying a variety of methods, finally distilled the soil with dilute HCl under reduced pressure at 50°C. for 20 minutes. He claimed that under these conditions no decomposition of organic matter took place, and carbonates were readily decomposed. Marr at the same time confirmed the conclusion of Amos (2) that occlusion of carbon dioxide in air-dry soil did not take place to any extent; this is also in accordance with our observations. MacIntire and Willis (8) suggested H_3PO_4 for the acid, but re-

[1] Contribution from the Department of Chemistry,

turned later to HCl (9) as the phosphoric acid failed to give complete decomposition of all carbonates in 30 minutes. 1:10 HCl was recommended by them for most soils and 1:5 for soils with greater than 15 per cent $CaCO_3$. They state "We have found that the action of 1:10 HCl on soil organic matter at room temperature is altogether negligible upon 5, 10, 20 gm. charges of the average soil." They also observe that if residual magnesite occurs in a soil "neither boiling for 1 minute, or several minutes, nor the Marr method, nor the Tennessee Station method will effect the complete decomposition of the mineral magnesium carbonate." Such soils are of rare occurrence, but they needed boiling for 30 minutes to get complete decomposition.

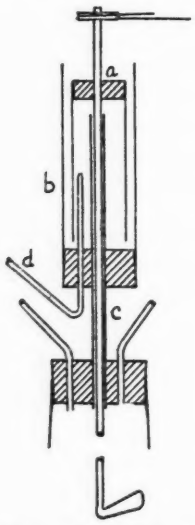

FIG. 1. STIRRER WITH MERCURY SEAL

MacIntire and Willis noted the necessity for shaking the soil with the acid and devised a multiple shaking apparatus which was later adopted by the Association of Official Agricultural Chemists. Truog (17) avoided the shaking by bubbling the CO_2-free air through the soil solution. In a previous report (6) we have noted the fineness to which soil is brought by continuous stirring, and in place of shaking we therefore introduced a stirrer combined with the mercury seal.

The stirrer with mercury seal was described by Brühl (4) and has become increasingly useful to the organic chemist (1). It can be applied frequently to soil problems. Figure 1 shows the stirring rod attached by a stopper (*a*) to the inverted tube which revolves with it. This tube is sealed to the passage of gas by the mercury in the outer tube (*b*). The tube (*c*) connects the seal

with the flask; the stirring rod, slightly greased, fits fairly closely into this. It is convenient to attach one of the small 1/8 H.P. stirring motors directly to the stirring rod to avoid vibration. An innovation is the presence of the extra inlet (*d*) which passes inside the revolving tube and terminates above the level of the mercury; this enables air to be drawn through the seal, effectively preventing any carbon dioxide from staying in the air spaces.

Preliminary stirring is unnecessary: a soil which was stirred with water free from carbon dioxide for 5 hours before adding the HCl, did not give any increase in the final amount of carbon dioxide compared to the same soil when stirred only during the time of aspiration. The speed of evolution of the gas without preliminary stirring was equal to the rate of production from the soil which had been previously stirred, indicating that the stirring during aspiration allows the acid to attack the carbonates with rapidity, as well as holding to a minimum the solution of carbon dioxide in the liquid (8). We have used a concentration of acid sufficient to make a 1:10 HCl in the flask when added to the soil and water. For soils with a high concentration of carbonates, or for lime materials, we have included sufficient acid to obtain approximately 1:10 HCl in the flask after the reaction, and have added the acid slowly.

A solution of potassium hydroxide is effective when used as the absorbent for the carbon dioxide but it necessitates slow aspiration. In order to accelerate the passage of the air, ascarite has been utilized. This sodium hydroxide, asbestos mixture was introduced in work on steel by Stetser and Norton (15) who found that the ascarite retained the water produced by the absorption of the carbon dioxide. Marsh (11), however, reported against its use for soils. He states that gases must be drawn or forced through the absorbing medium for periods varying from 12 to 48 hours or more, and as the ascarite lost water after 5 hours, he concluded that "where long runs are necessary as in the determination of CO_2 production from soil, ascarite because of loss of moisture, can not be successfully used."

A possible means of adapting ascarite for soils was pointed out by Underwood (18). In the direct determination of carbon dioxide in limestone Underwood passed the gas over phosphorus pentoxide before it entered a Midvale absorption bulb containing the ascarite, and again over the pentoxide before it left the bulb. In this way he took care of any excess moisture which was not absorbed by the ascarite. We have used a U tube with glass stopcocks for the absorbing materials. Approximately one-half inch of phosphorus pentoxide is placed on glass wool on the ascarite where the gases emerge from the tube. This allows of much faster aspiration than if a potassium hydroxide solution is used, and it is easy to see when the material should be renewed.

A drying agent, which compares well with phosphorus pentoxide in absorptive capacity and has several advantages, is magnesium perchlorate trihydrate, sold under the name "dehydrite." This was tested by Willard and Smith (19) and has been used by a number of investigators for carbon determinations

(3, 14); it has been applied by Lee and Brown (7) to biological processes. The dehydrite can be used until it increases in weight 20 to 25 per cent, and does not become sticky when charging the tubes. It gives little resistance to the gas and when used shrinks so that it does not stick or clog. This can replace the phosphorus pentoxide, but we have used a somewhat larger quantity of the dehydrite in the weighing tube.

In order to facilitate the removal of the tube from the train, the arms may be ground to make glass joints, instead of being attached with rubber connec-

TABLE 1

Weight of U tubes without aspiration

	MINUTES IN BALANCE CASE		
	5	15	25
Wiped with alcohol, etc.	82.8876	82.8877	82.8880
Repeated	82.8876	82.8879	82.8880
Repeated	82.8876	82.8876	82.8881
Wiped with alcohol, etc.	82.8879	82.8881	82.8882
Repeated	82.8878	82.8881	82.8882
Wiped with alcohol, etc.	34.8027	34.8028	34.8029
Repeated	34.8026	34.8028	34.8029
Repeated	34.8026	34.8028	34.8029

TABLE 2

Weight of U tubes with aspiration

TREATMENT	HOURS ASPIRATED	MINUTES IN BALANCE CASE			
		5	10	15	20
Preliminary	1.25	60.2356	60.2358	60.2360	60.2360
HCl added	1.25	60.2683	60.2682	60.2682	60.2683
	1.5	60.2685	60.2685	60.2688	60.2688
	4.5	60.2696	60.2695	60.2696	60.2696
	1.0	60.2696	60.2698	60.2699	60.2699
$CaCO_3$ *per cent*		0.515	0.515	0.514	0.514

tions. The U tube is wiped off with an alcohol-dampened cloth and afterwards with a dry cloth: it is then placed in the balance case for a few minutes before weighing. We have found, even with a large U tube weighing 80 gm. when filled, that the original weight rarely changes more than 0.2 to 0.3 mgm. when it is left in the laboratory air for the five or six hours necessary for aspiration. Tubes weighing from 40 to 60 gm. when packed with the ascarite and P_2O_5 or dehydrite give a volume of sufficient size for a large number of

soil determinations; weighing with a tare is generally unnecessary if tubes are wiped off and weighed at the beginning of the run, although the weight will alter on long standing. Table 1 gives figures for both large and small tubes after being wiped with alcohol. Weights were standardized and weighings made as outlined by Richards (13) and are reported to the nearest 0.1 mgm.

From the figures given it will be seen that the gain in weight is very slow after 5 minutes in the balance case. Table 1 shows that consistent results can be obtained by wiping with the alcohol-dampened cloth, followed by a dry cloth and weighing after 5 minutes. Pregl (12) suggests the final wiping should be with chamois skin in microanalytical work in order to prevent electrification, and this procedure can be recommended. Table 2 gives figures for an actual series of weighings during a run on soil, with the CO_2 calculated as $CaCO_3$. A sample of Iceland Spar gave the following results: weight after

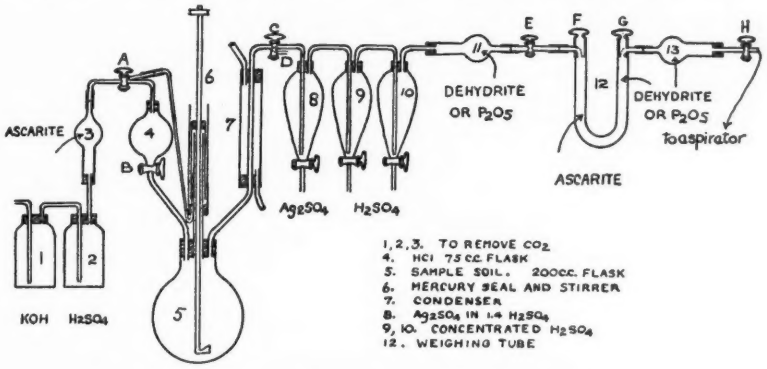

FIG. 2. APPARATUS FOR CARBON DIOXIDE DETERMINATION IN SOILS

5 minutes, 99.85 per cent $CaCO_3$; after 10 minutes, 99.84; 15 minutes, 99.85; 20 minutes, 99.86.

In figure 2, numbers 1, 2, and 3 serve to remove carbon dioxide from the air. The dropping funnel 4 is fitted with a rubber stopper, and is filled completely with the hydrochloric acid. A small water condenser, made of two glass tubes, has been found satisfactory for substances which do not need boiling; this protects from too much dilution the silver sulfate which is to catch the hydrochloric acid. When a silver precipitate forms it is easily removed through the stopcock at the bottom, and by creating a slight vacuum, a fresh solution is sucked up. The concentrated sulfuric in the following funnels can be replaced in the same way. The glass tubes in these are drawn to fine points, as in numbers 1 and 2, to decrease the size of the bubbles. Under the conditions the phosphorus pentoxide or dehydrite in 11 lasts for a long time without renewal. If the absorption tube 12 is fastened with rubber connections instead of ground glass joints, care must be taken that small

particles of rubber do not get inside the arms. Other joints may be wiped with benzene saturated with equal parts of paraffin and beeswax.

PROCEDURE

From 5 to 20 gm. of soil which has been passed through a 1-mm. sieve, is placed in flask 5 and washed down with a little CO_2-free water. Air is then drawn through the apparatus by running water from the aspirator; by the use of the three-way stopcock at *A* the CO_2-free air can be taken through the

TABLE 3

Carbon dioxide from soil, calcium carbonate and mixtures

SAMPLE	TEMPERATURE	WEIGHT OF SOIL	WEIGHT OF $CaCO_3$	ASPIRATION TO CONSTANT WEIGHT		WEIGHT OF CO_2	$CaCO_3$ IN SAMPLE	$CaCO_3$ IN SOIL
		gm.	*gm.*	*hours*	*liters*	*gm.*	*per cent*	*per cent†*
Iceland spar	Room	.....	1.8171	5	15	0.7978	99.85	.....
Iceand spar	Room	.....	1.9912	4	13	0.8748	99.92	.....
Iceland spar	Room	.....	1.2210	4.5	14	0.5373	100.00	.....
Iceland spar	Room	.....	1.9414	3.75	14	0.8534	99.98	.....
						Average	99.94	
Soil 10	Room	5.0000	.....	5.25	14	0.1305	5.94	.....
Soil + spar	Room	5.0000	1.6219	4.5	19	0.8431	.....	5.93
Soil 10	50°	5.0000	.....	3.25*	9	0.1318	6.00	.....
Soil 22c	Room	10.0000	.....	4	13.5	0.4898	11.14	.....
Soil 22c + spar	Room	10.0000	1.2430	6	17	1.0357	.....	11.13
Soil 22c	50°	10.0000	.....	4*	12	0.4942	11.24	.....
Soil 22c + spar	50°	10.0000	1.2430	1.25*	5	1.0373	.....	11.17
$CaCO_3$	Room	.....	0.3276	5.5	24	0.1398	97.05	.....
Soil 32	Room	15.0000	.....	7.25	20	0.0340	0.52	.....
Soil 32 + $CaCO_3$	Room	15.0000	0.2757	5.75	32	0.1511	.....	0.51
Soil 32	50°	15.0000	.....	7.25*	16	0.0356	0.54	.....
Soil 32 + $CaCO_3$	50°	15.0000	0.2757	3.00*	14	0.1520	.....	0.53

* After aspiration at room temperature as above. Final figures were the same when soil was run at 50° from the time of adding acid.

† Total in mixture minus amount in $CaCO_3$ as found at room temperature.

stirrer or through the funnel 4. If there are no leaks, when the inlet at 1 is closed for a few minutes, the water will cease to flow. Stopcocks are then closed and tube 12 is weighed as described, and the aspiration repeated until the weight is constant. The tube is replaced in the train and funnel 4 filled with the dilute hydrochloric acid. Before opening *B* stirring and aspiration are started, and then *A* and *B* turned to allow a few drops at a time to pass into flask 5. When all the acid is in the flask, aspiration proceeds slowly at first, and is gradually increased and continued until no change in weight in

the absorption bulb occurs. The CO_2-free air is drawn through the mercury seal or through funnel 4 as desired. Between runs the stopcocks are closed, so that the drying parts of the system 8, 9, 10, and 11 are kept free from the outside air. After the introduction of the soil into 5, the three-way stopcock may be opened at *D* and air drawn through from 1 to *D*; this materially accelerates the preliminary removal of the CO_2 from the apparatus.

The three soils reported contain very widely differing amounts of carbonate. It will be seen from table 3 that in the mixture of soil and carbonate, when the CO_2 in the Iceland spar is subtracted from the mixture, the recovery of the CO_2 from the soil is almost identically the same as it is when the soil only is used. At 50° there is a slight increase in the amount of CO_2 given off. MacIntire and Willis ascribe this to a decomposition of the organic matter at the higher temperature (8), and this seems to be the case. Saturated CO_2 water with HCl was aspirated and all the CO_2 was removed in about 2 hours at room temperature. No increase in weight was found by raising the temperature to 50°. Similarly the Iceland spar showed no increase in the weight of CO_2 on running at 50°. The indication is that the increase when soil is present is due to the action on the soil and not to any CO_2 dissolved in the water.

During the time of aspiration little or no attention is needed —five to seven hours has been found long enough in almost all cases for the U tube to come to constant weight.

SUMMARY

1. An improved method for the gravimetric determination of soil carbonates has been outlined.

2. The method makes use of ascarite and either dehydrite or phosphorus pentoxide as absorbents.

3. The soil mixture is stirred instead of shaken, and the mercury seal is adapted for this purpose.

4. One to ten hydrochloric acid is used at room temperature. At 50° there is a slight increase in the amount of CO_2 given off.

5. Figures are given for determinations on three soils containing widely differing amounts of carbonates, both alone and mixed with calcium carbonate.

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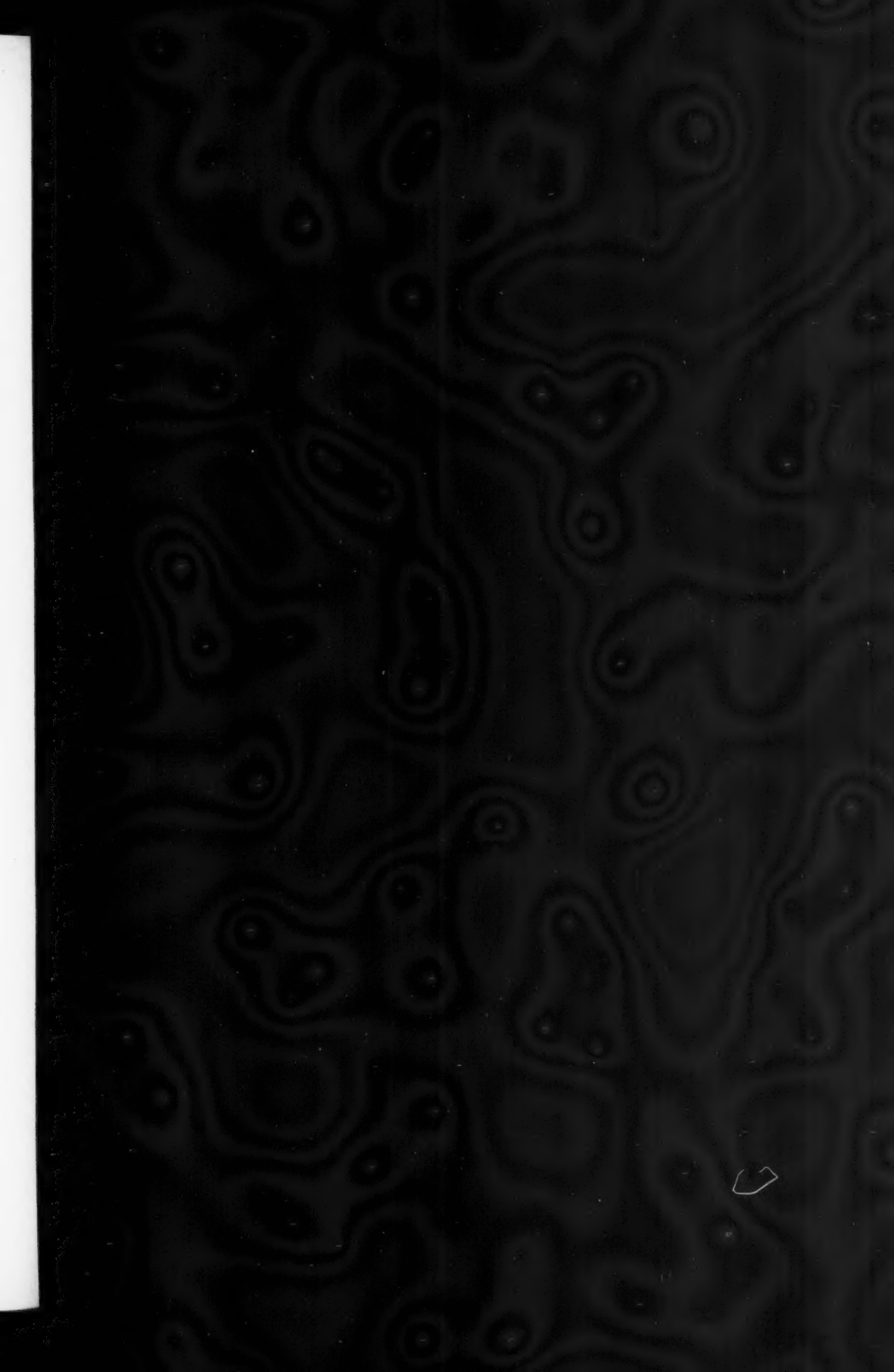
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